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NEWS	21	MAR 22	LWPI reloaded
NEWS	22	MAR 30	RDISCLOSURE reloaded with enhancements
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L1     9610682 TREATMENT

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L2      259815 L1 AND INHIBIT

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L3         635 L2 AND NEPHRITIS

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L4         288 L3 AND ANTIBOD?

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L5           0 L4 AND PDGF-D

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L7           6 DUP REMOVE L6 (3 DUPLICATES REMOVED)

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L7   ANSWER 1 OF 6  SCISEARCH  COPYRIGHT (c) 2007 The Thomson Corporation  on
     STN
2004:222461   The Genuine Article (R) Number: 777WH. New perspectives in
     treatment of glomerulonephritis.  Coppo R (Reprint); Amore A.
     Osped Infantile Regina Margherita, Nephrol Dialysis & Transplantat Dept,
     Piazza Polonia 94, I-10126 Turin, Italy (Reprint); Osped Infantile Regina
     Margherita, Nephrol Dialysis & Transplantat Dept, I-10126 Turin, Italy;
     Regina Margherita Childrens Univ Hosp, Nephrol Dialysis & Transplantat
     Dept, Turin, Italy. PEDIATRIC NEPHROLOGY (MAR 2004) Vol. 19, No. 3, pp.
     256-265. ISSN: 0931-041X. Publisher: SPRINGER-VERLAG, 175 FIFTH AVE, NEW
     YORK, NY 10010 USA. Language: English.
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB        In chronic glomerulonephritis (GN) the development of the tissue
     damage and progression to fibrosis is related to the individual immune
     response which brings about excessive inflammation, failure to activate
     regression and glomerular repair and excessive fibrogenic activity.
     Therefore, the present standard treatment of GN has two aims, to
     fight the acute inflammation and to inhibit the progressive
     renal fibrosis.  New avenues in the anti-inflammatory and
     immunosuppressive treatment of the active phase of glomerular
     diseases include the use of drugs proven to be of value in organ
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transplantation (mycophenolate mofetil, rapamycin or anti-immune adhesion and anti-co-stimulatory molecules). Interest has recently focused on anti-inflammatory cytokines (monoclonal antibodies, peptidic antagonists or anti-sense oligonucleotides against TNF-alpha, anti-PDGF-beta, anti-TGF-beta and cytokine receptor antagonists) and anti-inflammatory natural cytokines (such as IL4, IL10, IL13 or low doses of TGFbeta). Other drugs may act by depleting B cells (such as anti-CD20 monoclonal antibody) or on several immune pathways, such as thalidomide or anti-cyclooxygenase 2. Several anti-sclerogenic drugs are already used for treatment of the chronic phase of glomerular diseases, such as antagonists of angiotensin II, statins and antioxidants. Other drugs are still experimental, including endothelin receptor antagonists and neutral endopeptidase or vasopeptidase inhibitors and other drugs operating on extracellular matrix accumulation/degradation mechanisms, e.g., pirfenidone. There are extremely interesting developments concerning activators of endogenous anti-inflammatory mechanisms, such as those regulated by peroxisome proliferator activated receptors. There is a need for successful treatment of chronic GN in childhood. This short review of the most promising new drugs shows there is reason to believe that the next decade will provide exciting new tools for the treatment of these diseases in children.

L7 ANSWER 2 OF 6 MEDLINE on STN DUPLICATE 1

1999341256. PubMed ID: 10412745. Effect of simvastatin on proliferative nephritis and cell-cycle protein expression. Yoshimura A; Nemoto T; Sugenoya Y; Inui K; Watanabe S; Inoue Y; Sharif S; Yokota N; Uda S; Morita H; Ideura T. (Department of Medicine, Showa University Fujigaoka Hospital, Yokohama, Japan.) Kidney international. Supplement, (1999 Jul) Vol. 71, pp. S84-7. Ref: 7. Journal code: 7508622. ISSN: 0098-6577. Pub. country: United States. Language: English.

AB BACKGROUND: Mesangial cell proliferation is important in subsequent mesangial matrix expansion in glomerular injury. Therefore, the regulation of mesangial cell proliferation may be critical in the treatment of glomerulonephritis. Inhibition of 3-hydro-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibits the production of mevalonate and has been shown to suppress proliferation in many cell types, including mesangial cells in vitro. It is expected that HMG-CoA reductase inhibitor may suppress mesangial cell proliferation and subsequent progression of glomerulonephritis. Recently, the tight relationship between cell-cycle regulatory protein expression and mesangial cell proliferation in experimental glomerulonephritis was demonstrated. The aim of the present study is to examine the effect of simvastatin, one of the HMG-CoA reductase inhibitors, on the glomerular cell proliferation and on the expression of CDK2 or p27Kip1 in mesangial cells in experimental glomerulonephritis in vivo. METHODS: The effect of simvastatin on a rat mesangial proliferative glomerulonephritis induced by antithymocyte antibody (anti-Thy 1.1 GN) was studied. Administration of simvastatin or vehicle (for control GN) were started from two days before disease induction, and was continued to the day of nephrectomy. Nephrectomy was done at days 0, 2, 4, 7, 12 and 20 after disease induction. Immunohistochemistry for proliferating cells, macrophages, alpha-smooth muscle actin, type IV collagen and PDGF -B chain was performed, respectively, in addition to conventional periodic-acid Schiff staining. Double immunostaining for CDK2/OX-7 or p27Kip1/OX-7 was also done, respectively. RESULTS: There was no difference in the degree of the initial injuries between simvastatin-treated and control GN rats. The most pronounced feature of simvastatin-treated GN was the suppression of the early glomerular cell proliferation (about 70% of proliferation was suppressed at day 4). At day 4, alpha-smooth muscle actin expression was also decreased in simvastatin-treated GN rats. Inhibition of macrophage recruitment into glomeruli by simvastatin was also a prominent feature (about 30% decrease in the number of glomerular macrophages at day 2). Simvastatin significantly suppressed subsequent mesangial matrix expansion and type IV collagen accumulation in glomeruli. Although it might simply reflect the

reduction in mesangial cells, glomerular PDGF-B chain expression was reduced. There was no significant difference in plasma lipids levels at day 2 and day 4. In vehicle-treated GN rats, the number of CDK2+/OX-7+ cells (CDK2-expressed mesangial cells) in glomeruli increased significantly from day 4 to day 7. Although simvastatin suppressed mesangial cell proliferation, the increase in the number of glomerular CDK2+/OX-7+ cells was also attenuated by simvastatin treatment. There was no difference in the number of p27Kip1+/OX-7+ cells (p27Kip1-expressed mesangial cells) in the glomerulus between vehicle-treated and simvastatin-treated GN rats. CONCLUSION: Simvastatin suppressed mesangial cell proliferation and subsequent matrix expansion, and macrophage infiltration into glomeruli in anti-Thy 1.1 GN rats. The antiproliferative effect of simvastatin in this model was also associated with the reduction of CDK2 expression in mesangial cells.

L7 ANSWER 3 OF 6 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1999:483481 The Genuine Article (R) Number: 209ZZ. Effect of simvastatin on proliferative nephritis and cell-cycle protein expression.
Yoshimura A (Reprint); Nemoto T; Sugenoya Y; Inui K; Watanabe S; Inoue Y; Sharif S; Yokota N; Uda S; Morita H; Ideura T. Showa Univ, Fujigaoka Hosp, Dept Med, Div Nephrol, Aoba Ku, 1-30 Fujiaoka, Yokohama, Kanagawa 2278501, Japan (Reprint); Showa Univ, Fujigaoka Hosp, Dept Med, Div Nephrol, Aoba Ku, Yokohama, Kanagawa 2278501, Japan. KIDNEY INTERNATIONAL (JUL 1999) Vol. 56, Supp. [71], pp. S84-S87. ISSN: 0085-2538. Publisher: BLACKWELL SCIENCE INC, 350 MAIN ST, MALDEN, MA 02148 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background. mesangial cell proliferation is important in subsequent mesangial matrix expansion in glomerular injury. Therefore, the regulation of mesangial cell proliferation may be critical in the treatment of glomerulonephritis. Inhibition of 3-hydro-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibits the production of mevalonate and has been shown to suppress proliferation in many cell types, including mesangial cells in vitro. It is expected that HMG-CoA reductase inhibitor may suppress mesangial cell proliferation and subsequent progression of glomerulonephritis. Recently, the tight relationship between cell-cycle regulatory protein expression and mesangial cell proliferation in experimental glomerulonephritis was demonstrated, The aim of the present study is to examine the effect of simvastatin, one of the HMG-CoA reductase inhibitors, on the glomerular cell proliferation and on the expression of CDK2 or p27Kip1 in mesangial cells in experimental glomerulonephritis in vivo.

Methods. The effect of simvastatin on a rat mesangial proliferative glomerulonephritis induced by antithymocyte antibody (anti-Thy 1.1 GN) was studied. Administration of simvastatin or vehicle (for control GN) were started from two days before disease induction, and was continued to the day of nephrectomy. Nephrectomy was done at days 0, 2, 4, 7, 12 and 20 after disease induction. Immunohistochemistry for proliferating cells. macrophages, or-smooth muscle actin, type IV collagen and PDGF-B chain was performed, respectively, in addition to conventional periodic-acid Schiff staining. Double immunostaining for CDK2/OX-7 or p27Kip1/OX-7 was also done, respectively.

Results. There was no difference in the degree of the initial injuries between simvastatin-treated and control GN rats. The most pronounced feature of simvastatin-treated GN was the suppression of the early glomerular cell proliferation (about 70% of proliferation was suppressed at day 4). At day 4, alpha-smooth muscle actin expression was also decreased in simvastatin-treated GN rats. Inhibition of macrophage recruitment into glomeruli by simvastatin was also a prominent feature (about 30% decrease in the number of glomerular macrophages at day 2), Simvastatin significantly suppressed subsequent mesangial matrix expansion and type IV collagen accumulation in glomeruli. Although it might simply reflect the reduction in mesangial cells. glomerular PDGF-B chain expression was reduced. There was no significant difference in plasma lipids levels at day 2 and day 4. In vehicle-treated GN rats, the

number of CDK2+/OX-7+ cells (CDK2-expressed mesangial cells) in glomeruli increased significantly from day 4 to day 7. Although simvastatin suppressed mesangial cell proliferation, the increase in the number of glomerular CDK2+/OX7+ cells was also attenuated by simvastatin treatment, There was no difference in the number of p27Kip1 +/OX-7+ cells (p27Kip1-expressed mesangial cells) in the glomerulus between vehicle-treated and simvastatin-treated GN rots.

Conclusion. Simvastatin suppressed mesangial cell proliferation and subsequent matrix expansion, and macrophage infiltration into glomeruli in anti-Thy 1.1 GN rats. The antiproliferative effect of simvastatin in this model was also associated with the reduction of CDK2 expression in mesangial cells.

L7 ANSWER 4 OF 6 EMBASE DUPLICATE 2

1999229512 EMBASE Effect of simvastatin on proliferative nephritis and cell-cycle protein expression. Yoshimura A.; Nemoto T.; Sugenoya Y.; Inui K.; Watanabe S.; Inoue Y.; Sharif S.; Yokota N.; Uda S.; Morita H.; Ideura T.. Dr. A. Yoshimura, Department of Medicine, Division of Nephrology, Showa University, 1-30 Fujigaoka, Aoba-ku, Yokohama 227-8501, Japan. Kidney International, Supplement Vol. 56, No. 71, pp. S84-S87 1999.

Refs: 10.

ISSN: 0098-6577. CODEN: KISUDF

Pub. Country: United States. Language: English. Summary Language: English. Entered STN: 19990715. Last Updated on STN: 19990715

AB Background. Mesangial cell proliferation is important in subsequent mesangial matrix expansion in glomerular injury. Therefore, the regulation of mesangial cell proliferation may be critical in the treatment of glomerulonephritis. Inhibition of 3-hydro-3-methylglutaryl coenzyme A (HMG- CoA) reductase inhibits the production of mevalonate and has been shown to suppress proliferation in many cell types, including mesangial cells in vitro. It is expected that HMG-CoA reductase inhibitor may suppress mesangial cell proliferation and subsequent progression of glomerulonephritis. Recently, the tight relationship between cell-cycle regulatory protein expression and mesangial cell proliferation in experimental glomerulonephritis was demonstrated. The aim of the present study is to examine the effect of simvastatin, one of the HMG-CoA reductase inhibitors, on the glomerular cell proliferation and on the expression of CDK2 or p27Kip1 in mesangial cells in experimental glomerulonephritis in vivo. Methods. The effect of simvastatin on a rat mesangial proliferative glomerulonephritis induced by antithymocyte antibody (anti-Thy 1.1 GN) was studied. Administration of simvastatin or vehicle (for control GN) were started from two days before disease induction, and was continued to the day of nephrectomy. Nephrectomy was done at days 0, 2, 4, 7, 12 and 20 after disease induction. Immunohistochemistry for proliferating cells, macrophages, α- smooth muscle actin, type IV collagen and PDGF-B chain was performed, respectively, in addition to conventional periodic-acid Schiff staining. Double immunostaining for CDK2/OX-7 or p27Kip1/OX-7 was also done, respectively. Results. There was no difference in the degree of the initial injuries between simvastatin-treated and control GN rats. The most pronounced feature of simvastatin-treated GN was the suppression of the early glomerular cell proliferation (about 70% of proliferation was suppressed at day 4). At day 4, α-smooth muscle actin expression was also decreased in simvastatin- treated GN rats. Inhibition of macrophage recruitment into glomeruli by simvastatin was also a prominent feature (about 30% decrease in the number of glomerular macrophages at day 2). Simvastatin significantly suppressed subsequent mesangial matrix expansion and type IV collagen accumulation in glomeruli. Although it might simply reflect the reduction in mesangial cells, glomerular PDGF-B chain expression was reduced. There was no significant difference in plasma lipids levels at day 2 and day 4. In vehicle-treated GN rats, the number of CDK2+/OX-7+ cells (CDK2-expressed mesangial cells) in glomeruli increased

significantly from day 4 to day 7. Although simvastatin suppressed mesangial cell proliferation, the increase in the number of glomerular CDK2+/OX-7+ cells was also attenuated by simvastatin treatment. There was no difference in the number of p27Kip1+/OX-7+cells (p27Kip1-expressed mesangial cells) in the glomerulus between vehicle-treated and simvastatin-treated GN rats. Conclusion. Simvastatin suppressed mesangial cell proliferation and subsequent matrix expansion, and macrophage infiltration into glomeruli in anti-Thy 1.1 GN rats. The antiproliferative effect of simvastatin in this model was also associated with the reduction of CDK2 expression in mesangial cells.

L7 ANSWER 5 OF 6 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1998:816569 The Genuine Article (R) Number: 130YD. Simvastatin suppresses glomerular cell proliferation and macrophage infiltration in rats with mesangial proliferative nephritis. Yoshimura A (Reprint); Inui K; Nemoto T; Uda S; Sugenoya Y; Watanabe S; Yokota N; Taira T; Iwasaki S; Ideura T. Showa Univ, Fujigaoka Hosp, Dept Med, Div Nephrol, Aoba Ku, 1-30 Fujigaoka, Yokohama, Kanagawa 227, Japan (Reprint); Showa Univ, Fujigaoka Hosp, Dept Med, Div Nephrol, Aoba Ku, Yokohama, Kanagawa 227, Japan. JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY (NOV 1998) Vol. 9, No. 11, pp. 2027-2039. ISSN: 1046-6673. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Inhibition of 3-hydro-3-methylglutaryl coenzyme A reductase inhibits the production of mevalonate and has been shown to suppress proliferation in many cell types. Therefore, 3-hydro-3-methylglutaryl coenzyme A reductase inhibitors may have a beneficial effect in glomerular disease, because glomerular cell proliferation is a central feature in the active glomerular injury. This study examines the effect of simvastatin on glomerular pathology in a rat mesangial proliferative glomerulonephritis (GN) induced by anti-thymocyte antibody (anti-Thy1.1 GN). There was no difference in the degree of the antibody and complement-mediated initial injuries between simvastatin-treated and control GN rats. The most pronounced feature of simvastatin-treated GN was the suppression of the early glomerular cell proliferation. The proliferative activity was maximal at day 4 after disease induction (26.5 +/- 7.0 of proliferating cell nuclear antigen-positive cells/glomerulus); however, approximately 70% of proliferation was suppressed by simvastatin treatment. At day 4 after disease induction, simvastatin administration also decreased alpha-smooth muscle actin expression in the glomerulus, which is a marker for mesangial cell activation. Inhibition of monocyte/macrophage recruitment into glomeruli by simvastatin was also a prominent feature. There was a 30% decrease in the number of glomerular ED-1(+) cells by simvastatin treatment at day 2 after disease induction. Furthermore, simvastatin remarkably suppressed subsequent mesangial matrix expansion and type IV collagen accumulation in glomeruli. We also found that the platelet-derived growth factor expression was reduced in simvastatin-treated nephritic rats, which might simply reflect the reduction in mesangial cell proliferation and mesangial cellularity. There was no significant difference in plasma cholesterol or triglyceride levels between simvastatin- and vehicle-treated nephritic rats at day 2 and day 4, which corresponded to the times when simvastatin treatment resulted in a reduction in mesangial cell proliferation. In conclusion, this is the first report to find that mesangial cell proliferation and matrix expansion have been blocked by simvastatin in vivo. The protective effect of simvastatin in the matrix expansion in anti-Thy1.1 GN was partly by inhibition of mesangial cell proliferation and monocyte/ macrophage recruitment into glomeruli, which were independent of a change in circulating lipids.

L7 ANSWER 6 OF 6 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 3

93162844 EMBASE Document No.: 1993162844. Heparin suppresses mesangial cell

proliferation and matrix expansion in experimental mesangioproliferative glomerulonephritis. Floege J.; Eng E.; Young B.A.; Couser W.G.; Johnson R.J.. Division of Nephrology, University of Washington,Seattle, WA 98195, United States. Kidney International Vol. 43, No. 2, pp. 369-380 1993. ISSN: 0085-2538. CODEN: KDYIA5
Pub. Country: United States. Language: English. Summary Language: English. Entered STN: 930704. Last Updated on STN: 930704

AB Proliferation and extracellular matrix (ECM) overproduction by glomerular mesangial cells characterizes many types of glomerulonephritis and often precedes the development of glomerulosclerosis. Heparin is a potent inhibitor of mesangial cell growth in vitro. We examined whether standard heparin can inhibit mesangial cell proliferation in vivo in the mesangioproliferative anti-Thy 1.1 nephritis. Untreated control rats were compared to rats infused with heparin either early (day -2 to 1) or late (day 2 to 5) after induction of anti-Thy 1.1 nephritis. The results show that heparin treatment significantly reduced mesangial cell proliferation regardless of when it was initiated. Heparin (either early or late treatment) also reduced mesangial basic fibroblast growth factor (bFGF) expression and platelet-derived growth factor (PDGF) receptor up-regulation as reflected by immunostaining, whereas PDGF B-chain expression was reduced only by late heparin treatment. Furthermore, heparin treatment markedly inhibited the mesangial matrix expansion for a variety of ECM proteins, including laminin, type I and IV collagen, fibronectin and entactin. Heparin did not affect the initial mesangiolysis, glomerular macrophage influx, deposition of anti-Thy 1.1 IgG or fibrinogen, or the glomerular platelet influx. These results suggest that heparin, via its antiproliferative rather than anticoagulant effect, can inhibit mesangial cell proliferation, overexpression of polypeptide growth factors, and ECM protein overproduction in vivo. The beneficial effect of heparin can be demonstrated even if treatment is initiated after the development of nephritis . By virtue of these properties, heparin may be an effective agent in the treatment of human mesangioproliferative disease and in the prevention of glomerulosclerosis.

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L9           46 L8 AND PDGF-D

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L10           6 L9 AND PDGF-DD

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L11            2 DUP REMOVE L10 (4 DUPLICATES REMOVED)

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L11  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2007 ACS on STN
2006:1206260   Document No. 145:500154  Methods and compositions for
     PDGF-D activation and inhibition.  Eriksson, Ulf (Ludwig
     Institute for Cancer Research, USA).  PCT Int. Appl. WO 2006122144 A2
     20061116, 30pp.  DESIGNATED STATES: W:  AE, AG, AL, AM, AT, AU, AZ, BA,
     BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC,
     EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG,
     KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN,
     MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE,
     SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU,
     ZA; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA,
     GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR.
     (English).  CODEN: PIXXD2.  APPLICATION: WO 2006-US17964 20060510.
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PRIORITY: US 2005-679233P 20050510.

AB The invention is based on the surprising discovery that urokinase plasminogen activator (uPA) cleaves and activates latent dimeric PDGF-DD. According to one aspect, the invention provides a method for inhibiting proteolytic processing of PDGF-D or PDGF-DD in a mammal in need thereof, comprising administering to the mammal an effective amount of uPA inhibitor. Preferably, the uPA inhibitor is an anti-uPA antibody, a PDGF-D CUB domain or a PDGF-DD CUB domain. The invention relates to methods for stimulating or inhibiting angiogenesis using pharmaceutical compns. comprising uPA or uPA agonists or uPA inhibitors. In another embodiment, a therapeutic method is provided for tumor or cancer treatment in a mammal, wherein the tumor is lined by or contains endothelial cells, the method comprising inhibiting proteolytic processing of PDGF-D or PDGF-DD in the mammal.

L11 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 1

2003398231. PubMed ID: 12937299. A fully human monoclonal antibody (CR002) identifies PDGF-D as a novel mediator of mesangioproliferative glomerulonephritis. Ostendorf Tammo; van Roeyen Claudia R C; Peterson Jeffrey D; Kunter Uta; Eitner Frank; Hamad Avin J; Chan Gerlinde; Jia Xiao-Chi; Macaluso Jennifer; Gazit-Bornstein Gadi; Keyt Bruce A; Lichenstein Henri S; LaRochelle William J; Floege Jurgen. (Division Nephrology, University of Aachen, Germany.) Journal of the American Society of Nephrology : JASN, (2003 Sep) Vol. 14, No. 9, pp. 2237-47. Journal code: 9013836. ISSN: 1046-6673. Pub. country: United States. Language: English.

AB PDGF-B is of central importance in mesangioproliferative diseases. PDGF-D, a new PDGF isoform, like PDGF-B, signals through the PDGF betabeta-receptor. The present study first determined that PDGF-D is mitogenic for rat mesangial cells and is not inhibited by a PDGF-B antagonist. Low levels of PDGF-D mRNA were detected in normal rat glomeruli. After induction of mesangioproliferative nephritis in rats by anti-Thy 1.1 mAb, glomerular PDGF-D mRNA and protein expression increased significantly from days 4 to 9 in comparison with nonnephritic rats. Peak expression of PDGF-D mRNA occurred 2 d later than peak PDGF-B mRNA expression. In addition, PDGF-D serum levels increased significantly in the nephritic animals on day 7. For investigating the functional role of PDGF-D, neutralizing fully human mAb were generated using the XenoMouse technology. Rats with anti-Thy 1.1-induced nephritis were treated on days 3 and 5 with different amounts of a fully human PDGF-DD -specific neutralizing mAb (CR002), equal amounts of irrelevant control mAb, or PBS by intraperitoneal injection. Specific antagonism of PDGF-D led to a dose-dependent (up to 67%) reduction of glomerular cell proliferation. As judged by double immunostaining for 5-bromo-2'-deoxyuridine and alpha-smooth muscle actin, glomerular mesangial cell proliferation was reduced by up to 57%. Reduction of glomerular cell proliferation in the rats that received CR002 was not associated with reduced glomerular expression of PDGF-B mRNA. PDGF-D antagonism also led to reduced glomerular infiltration of monocytes/macrophages (day 5) and reduced accumulation of fibronectin (day 8). In contrast, no effect was noted in normal rats that received an injection of CR002. These data show that PDGF-D is overexpressed in mesangioproliferative states and can act as an auto-, para-, or even endocrine glomerular cell mitogen, indicating that antagonism of PDGF-D may represent a novel therapeutic approach to mesangioproliferative glomerulonephritides.

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PROCESSING COMPLETED FOR L9
L12 25 DUP REMOVE L9 (21 DUPLICATES REMOVED)

=> d l12 1-25 cbib abs

L12 ANSWER 1 OF 25 MEDLINE on STN
2007249164. PubMed ID: 17308324. PDGF-D inhibition by CR002 ameliorates tubulointerstitial fibrosis following experimental glomerulonephritis. Boor Peter; Konieczny Andrzej; Villa Luigi; Kunter Uta; van Roeyen Claudia R C; Larochelle William J; Smithson Glennda; Arrol Sharon; Ostendorf Tammo; Floege Jurgen. (Division of Nephrology, University Hospital Aachen, Pauwelsstr. 30, D-52074 Aachen, Germany.. boor@email.cz) . Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association, (2007 May) Vol. 22, No. 5, pp. 1323-31. Electronic Publication: 2007-02-17. Journal code: 8706402. ISSN: 0931-0509. Pub. country: England: United Kingdom. Language: English.
AB BACKGROUND: Arresting or regressing kidney scarring is of major clinical relevance. Platelet-derived growth factor D (PDGF-D) is widely expressed in fibrotic kidneys. Administration of the PDGF-D neutralizing fully human monoclonal antibody CR002 in the acute phase of progressive anti-Thy 1.1 glomerulonephritis reduced glomerular and secondary tubulointerstitial damage. METHODS: Using this model, we now assessed the effects of CR002 (n = 15) vs irrelevant control IgG (n = 17) administered on days 17, 28 and 35 after disease induction, i.e. after acute glomerular damage had subsided. Results. In vitro, CR002 inhibited the PDGF-D- but not the PDGF-B-induced proliferation of rat renal fibroblasts. Following the first CR002 injection on day 17, exposure to therapeutic levels was maintained until day 49. Proteinuria in the CR002-treated group was transiently reduced between days 49 and 77 (-19 to -23% in comparison with the controls; P < 0.05). On day 100, CR002 treatment reduced the number of rats that had doubled their serum creatinine (CR002: 40 vs controls: 71%; P < 0.05). Compared with controls, the CR002 animals, on day 100, significantly lowered glomerular expression of vimentin and collagens as well as tubulointerstitial damage scores, interstitial fibrosis, vimentin and cortical PDGF-D mRNA levels. CONCLUSIONS: PDGF-D antagonism, even after the phase of acute glomerular damage, exerts beneficial effects on the course of tubulointerstitial damage, i.e. the final common pathway of most renal diseases.

L12 ANSWER 2 OF 25 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
2007:78334 Document No.: PREV200700078762. Platelet-derived growth factor D, DNA coding therefor, and uses thereof. Anonymous; Eriksson, Ulf [Inventor]; Aase, Karin [Inventor]; Li, Xuri [Inventor]; Ponten, Annica [Inventor]; Uutela, Marko [Inventor]; Alitalo, Kari [Inventor]; Oestman, Arne [Inventor]; Heldin, Carl-Henrik [Inventor]. Stockholm, Sweden. ASSIGNEE: Ludwig Institute for Cancer Research; Licentia Ltd. Patent Info.: US 07148037 20061212. Official Gazette of the United States Patent and Trademark Office Patents, (DEC 12 2006)
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.
AB PDGF-D, a new member of the PDGF/VEGF family of polypeptide growth factors, is described, as well as nucleotide sequences encoding, methods for producing, pharmaceutical compositions containing this new growth factor, and its antibodies and other antagonists. Also disclosed are transfected and transformed host cells expressing PDGF-D, and uses thereof in medical and diagnostic applications.

L12 ANSWER 3 OF 25 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
2007:22709 Document No.: PREV200700033358. Method for stimulating connective tissue growth or wound healing. Anonymous; Uutela, Marko [Inventor]; Eriksson, Ulf [Inventor]; Alitalo, Kari [Inventor]. University of Helsinki, Finland. ASSIGNEE: Ludwig Institute for Cancer Research; Licentia Ltd. Patent Info.: US 07105481 20060912. Official Gazette of the United States Patent and Trademark Office Patents, (SEP 12 2006)

CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB PDGF-D, a new member of the PDGF/VEGF family of growth factors, as well as the nucleotide sequence encoding it, methods for producing it, antibodies and other antagonists to it, transfected and transformed host cells expressing it, pharmaceutical compositions containing it, and uses thereof in medical and diagnostic applications, including methods for stimulating growth of a connective tissue or healing a wound in a mammal, which methods comprise administering to the mammal an effective amount of PDGF-D polypeptides or polynucleotides encoding the PDGF-D polypeptides.

L12 ANSWER 4 OF 25 CAPLUS COPYRIGHT 2007 ACS on STN

2006:1206260 Document No. 145:500154 Methods and compositions for PDGF-D activation and inhibition. Eriksson, Ulf (Ludwig Institute for Cancer Research, USA). PCT Int. Appl. WO 2006122144 A2 20061116, 30pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2006-US17964 20060510. PRIORITY: US 2005-679233P 20050510.

AB The invention is based on the surprising discovery that urokinase plasminogen activator (uPA) cleaves and activates latent dimeric PDGF-DD. According to one aspect, the invention provides a method for inhibiting proteolytic processing of PDGF-D or PDGF-DD in a mammal in need thereof, comprising administering to the mammal an effective amount of uPA inhibitor. Preferably, the uPA inhibitor is an anti-uPA antibody, a PDGF-D CUB domain or a PDGF-DD CUB domain. The invention relates to methods for stimulating or inhibiting angiogenesis using pharmaceutical compns. comprising uPA or uPA agonists or uPA inhibitors. In another embodiment, a therapeutic method is provided for tumor or cancer treatment in a mammal, wherein the tumor is lined by or contains endothelial cells, the method comprising inhibiting proteolytic processing of PDGF-D or PDGF-DD in the mammal.

L12 ANSWER 5 OF 25 MEDLINE on STN DUPLICATE 1

2006170498. PubMed ID: 16510766. Antagonism of PDGF-D by human antibody CR002 prevents renal scarring in experimental glomerulonephritis. Ostendorf Tammo; Rong Song; Boor Peter; Wiedemann Stefanie; Kunter Uta; Haubold Ulrike; van Roeyen Claudia R C; Eitner Frank; Kawachi Hiroshi; Starling Gary; Alvarez Enrique; Smithson Glennda; Floege Jurgen. (Division of Nephrology, University Hospital Aachen, Pauwelsstrasse 30, D-52074 Aachen, Germany.. tostendorf@ukaachen.de) . Journal of the American Society of Nephrology : JASN, (2006 Apr) Vol. 17, No. 4, pp. 1054-62. Electronic Publication: 2006-03-01. Journal code: 9013836. ISSN: 1046-6673. Pub. country: United States. Language: English.

AB Glomerular mesangial cell proliferation and/or matrix accumulation characterizes many progressive renal diseases. PDGF-D was identified recently as a novel mediator of mesangial cell proliferation in vitro and in vivo. This study investigated the long-term consequences of PDGF-D inhibition in vivo. Rats with progressive mesangioproliferative glomerulonephritis (uninephrectomy plus anti-Thy-1.1 antibody) received the PDGF-D -neutralizing, fully human mAb CR002 on days 3, 10, and 17 after disease induction. Glomerular mesangioproliferative changes on day 10 were significantly reduced by anti-PDGF-D treatment as compared with control antibody. Eight weeks after disease induction, anti-PDGF-D therapy significantly ameliorated focal segmental glomerulosclerosis, podocyte damage (de novo

desmin expression), tubulointerstitial damage, and fibrosis as well as the accumulation of renal interstitial matrix including type III collagen and fibronectin. Treatment with anti-PDGF-D also reduced the cortical infiltration of monocytes/macrophages on day 56, possibly related to lower renal cortical complement activation (C5b-9 deposition) and/or reduced epithelial-to-mesenchymal transition (preserved cortical expression of E-cadherin and reduced expression of vimentin and alpha-smooth muscle actin). In conclusion, these data provide evidence for a causal role of PDGF-D in the pathogenesis of renal scarring and point to a new therapeutic approach to progressive mesangioproliferative renal disease.

L12 ANSWER 6 OF 25 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 2

2006:671408 Document No.: PREV200600679854. Effect of late treatment with a PDGF-D monoclonal antibody in progressive mesangioproliferative glomerulonephritis. Boor, Peter [Reprint Author]; Konieczny, Andrzej; Villa, Luigi; Kunter, Uta; Smithson, Glennda; Floege, Juergen; Ostendorf, Tammo. RWTH Univ Aachen, Div Nephrol, Aachen, Germany. Nephrology Dialysis Transplantation, (JUL 2006) Vol. 21, No. Suppl. 4, pp. 34.
Meeting Info.: 43rd ERA-EDTA Congress. Glasgow, UK. July 15 -18, 2006. ERA; EDTA.
ISSN: 0931-0509. Language: English.

L12 ANSWER 7 OF 25 CAPLUS COPYRIGHT 2007 ACS on STN

2005:1028061 Document No. 143:299506 Method for stimulating connective tissue growth or wound healing using PDGF-D polypeptides or polynucleotides. Eriksson, Ulf; Uutela, Marko; Alitalo, Kari (Ludwig Institute for Cancer Research, USA). U.S. Pat. Appl. Publ. US 2005209136 A1 20050922, 75 pp., Cont.-in-part of U.S. Ser. No. 260,539. (English). CODEN: USXXCO. APPLICATION: US 2004-794392 20040308. PRIORITY: US 1998-107852P 19981110; US 1998-113997P 19981228; US 1999-150604P 19990826; US 1999-157108P 19991004; US 1999-157756P 19991005; US 1999-438046 19991110; US 2000-691200 20001019; US 2002-86623 20020304; US 2002-260539 20021001.

AB PDGF-D, a new member of the PDGF/VEGF family of growth factors, as well as the nucleotide sequence encoding it, methods for producing it, antibodies and other antagonists to it, transfected and transformed host cells expressing it, pharmaceutical compns. containing it, and uses thereof in medical and diagnostic applications, including methods for stimulating growth of a connective tissue or healing a wound in a mammal, which methods comprise administering to the mammal an effective amount of PDGF-D polypeptides or polynucleotides encoding the PDGF-D polypeptides.

L12 ANSWER 8 OF 25 MEDLINE on STN DUPLICATE 3

2005104059. PubMed ID: 15611105. Platelet-derived growth factor D, tissue-specific expression in the eye, and a key role in control of lens epithelial cell proliferation. Ray Sugata; Gao Chun; Wyatt Keith; Fariss Robert N; Bundek Amanda; Zelenka Peggy; Wistow Graeme. (NEI, National Institutes of Health, Bethesda, Maryland 20892-0703, USA.) The Journal of biological chemistry, (2005 Mar 4) Vol. 280, No. 9, pp. 8494-502. Electronic Publication: 2004-12-16. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Platelet-derived growth factor D (PDGF-D), also known as Iris-expressed growth factor, is a member of the PDGF/vascular endothelial growth factor family. The expression of PDGF-D in the eye is tissue-specific. In the anterior segment, it is localized to iris and ciliary body, whereas in the retina, PDGF-D is restricted to the outer plexiform layer. PDGF-D is present in aqueous humor but is not detectable in mature lens or in mouse lens-derived alphaTN4-1 cells. However, it is expressed in rabbit lens-derived N/N1003A cells. N/N1003A cell-conditioned medium

stimulates proliferation in rat lens explants, and this is blocked by immunodepletion of PDGF-D. Immunopurified PDGF-D also stimulates cell proliferation in rat lens explants and in NIH 3T3 cells. In organ culture of rat eye anterior segments, anti-PDGF-D strongly inhibits lens epithelial cell proliferation. This finding suggests a major in vivo role for PDGF-D in the mechanisms of coordinated growth of eye tissues. Intervention in the PDGF-D pathway in the eye, perhaps by antibody or blocking peptide, could be useful in the treatment of certain cataracts, including post-operative secondary cataract.

L12 ANSWER 9 OF 25 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
2004:232973 Document No.: PREV200400235441. Platelet-derived growth factor D. Eriksson, Ulf [Inventor, Reprint Author]; Aase, Karin [Inventor]; Lee, Xuri [Inventor]; Ponten, Annica [Inventor]; Uutela, Marko [Inventor]; Alitalo, Kari [Inventor]; Oestman, Arne [Inventor]; Heldin, Carl-Henrik [Inventor]. Stockholm, Sweden. ASSIGNEE: Ludwig Institute for Cancer Research; Helsinki University, Helsinki, Finland. Patent Info.: US 6706687 20040316. Official Gazette of the United States Patent and Trademark Office Patents, (Mar 16 2004) Vol. 1280, No. 3. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133 (ISSN print). Language: English.
AB PDGF-D, a new member of the PDGF/VEGF family of growth factors, is described, as well as the nucleotide sequence encoding it, methods for producing it, antibodies and other antagonists to it, transfected and transformed host cells expressing it, pharmaceutical compositions containing it, and uses thereof in medical and diagnostic applications.

L12 ANSWER 10 OF 25 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
2005:477354 Document No.: PREV200510269258. Cell-to-cell contact is critical for the survival of hematopoietic progenitor cells on osteoblasts. Wang, Jianhua [Reprint Author]; Taichman, Russell; Jung, Younghun; Havens, Aaron; Sun, Yanxi; Wang, Jingcheng; Jin, Taocong. Univ Michigan, Sch Dent, Ann Arbor, MI 48109 USA. Blood, (NOV 16 2004) Vol. 104, No. 11, Part 1, pp. 363A.
Meeting Info.: 46th Annual Meeting of the American-Society-of-Hematology. San Diego, CA, USA. December 04 -07, 2004. Amer Soc Hematol.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.
AB Osteoblasts constitute part of the stromal cell support system in marrow for hematopoiesis, however little is known as to how they interact with hematopoietic stem cells (HSCs). In vitro studies have demonstrated that the survival of HSCs in co-culture with osteoblasts requires intimate cell-to-cell contact. This suggests that the osteoblast-derived factor(s) that supports stem cell activities are either produced in very small quantities, are rapidly turned over, may be membrane-anchored and/or requires the engagement of cell-cell adhesion molecules yet to be determined. In the present report we found that survival of hematopoietic progenitor cells on osteoblasts is dependent upon the engagement of VLA-4 (alpha(4)beta(1)) and VLA-5 (alpha(1)beta(1)) receptors using function blocking antibodies. Surprisingly, cell-to-cell contact is not absolutely required to support progenitor activity, but does not require receptor-ligand engagement of the VLA-4 and LFA-1 complexes, which can in part be replaced through the use of recombinant ligands (fibronectin, ICAM-1, VCAM-1). Moreover conditioned once these receptors were engaged, medium derived from HSCs grown on osteoblasts ligands supported significantly greater hematopoietic progenitors in vitro than did osteoblast-conditionedor HSC-conditioned medium alone. As an initial attempt to identify the activity we examined which genes are activated following the establishment of osteoblast-CD34(+) cell co-cultures nine separate co-cultures were estabisilled and the RNA was pooled and analyzed on Affymetrix HG-U133A chips at 24 hours. Initially our analysis revealed that there were 259 genes that are up regulated at 24

hours, and 14 genes that are down regulated. Inspection revealedthat 30 of these signals were repeated at least once suggesting that 206 genuine gene candidates were differentially expressed resulting from the co-culture. A significant proportion of the differentially expressed cDNAs represent intracellular signaling ligands 16.5% (n=34) and cell surface receptors 13.5% (n=28). Molecules associated with assembly of the extra cellular matrix or its degradation comprised 7.2% n=15) of the differentially up regulated molecules. Molecules associated with intracellular signaling, novel sequences and intermediate metabolism comprised the majority of the remaining activities. Amoung the candidates of extra cellular signaling molecules, we noted that IL-6, LIF, MIP-1 alpha and SDF-1 were identified in the microarray analysis. This observation was most gratifying as we had previously reported that IL-6, LIF and MIP-1a activities are critical components of an HSC-osteoblast microenvironment. Other notable cytokine messages for BMP-2, CCL7, FGF2b, GRO1 alpha, GRO3, IGF1,IL1 beta, IL-8, IL-11, LIF, PDGF-D and the receptors for CCL7 (CCR7), Elevations in mRNA for fibronectin, lysine hydroxylase-like proteins, laminin and Type I collagen suggest that the presence of hematopoietic cells also induces osteoblastic activities. While the identity of those molecules present in the co-cultured medium remain to be identified, the data suggests that hematopoietic cells cooperate with osteoblasts to assemble the various marrow microenvironments by directing the synthesis of osteoblast-derived cytokines to improve HSC survival.

L12 ANSWER 11 OF 25 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2004:147051 The Genuine Article (R) Number: 769AQ. Exogenous PDGF-D is a potent mesangial cell mitogen and causes a severe mesangial proliferative glomerulopathy. Hudkins K L; Gilbertson D G; Carling M; Taneda S; Hughes S D; Holdren M S; Palmer T E; Topouzis S; Haran A C; Feldhaus A L; Alpers C E (Reprint). Univ Washington, Dept Pathol, Box 357470, Seattle, WA 98195 USA (Reprint); Univ Washington, Dept Pathol, Seattle, WA 98195 USA; Zymogenet Inc, Seattle, WA USA. JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY (FEB 2004) Vol. 15, No. 2, pp. 286-298. ISSN: 1046-6673. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The PDGF family consists of at least four members, PDGF-A, -B, -C, and -D. All of the PDGF isoforms bind and signal through two known receptors, PDGF receptor-alpha and PDGF receptor-beta, which are constitutively expressed in the kidney and are upregulated in specific diseases. It is well established that PDGF-B plays a pivotal role in the mediation of glomerular mesangial cell proliferation. However, little is known of the roles of the recently discovered PDGF-C and -D in mediating renal injury. In this study, adenovirus constructs encoding PDGF-B, -C, and -D were injected into mice. Mice with high circulating levels of PDGF-D developed a severe mesangial proliferative glomerulopathy, characterized by enlarged glomeruli and a striking increase in glomerular cellularity. The PDGF-B-overexpressing mice had a milder proliferative glomerulopathy, whereas the mice overexpressing PDGF-C and those that received adenovirus alone showed no measurable response. Mitogenicity of PDGF-D and -B for mesangial cells was confirmed in vitro. These findings emphasize the importance of engagement of PDGF receptor-P in transducing mesangial cell proliferation and demonstrate that PDGF-D is a major mediator of mesangial cell proliferation. Finally, this approach has resulted in a unique and potentially valuable model of mesangial proliferative glomerulopathy and its resolution.

L12 ANSWER 12 OF 25 CAPLUS COPYRIGHT 2007 ACS on STN

2003:551635 Document No. 139:99858 Antibodies directed to spinal cord-derived growth factor-B. Corvalan, Jose R. F.; Jia, Xiao-Chi; Feng, Xiao; Yang, Xiao-Dong; Chen, Francine; Gazit, Gadi; Weber, Richard; Bezabeh, Binyam (Abgenix, Inc., USA). PCT Int. Appl. WO 2003057857 A2

20030717, 256 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US398 20030106. PRIORITY: US 2002-41860 20020107.

AB The authors disclose fully human monoclonal antibodies directed to spinal cord-derived growth factor-B (SCDGFB/PDGFD). In one example, the antibodies are shown to neutralize the proliferative response of fibroblasts to a p53 fragment of SCDGFB/PDGFD. In a second example, the antibodies comprise reagents for the detection of SCDGFB/PDGFD in blood in various malignancies.

L12 ANSWER 13 OF 25 CAPLUS COPYRIGHT 2007 ACS on STN

2003:320042 Document No. 138:315099 Preparation and production of dimerized PDGF-D chains binding to the PDGF receptors α/β and β/β. Moore, Margaret D.; Fox, Brian A. (Zymogenetics, Inc., USA). PCT Int. Appl. WO 2003033677 A2 20030424, 47 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US33563 20021018. PRIORITY: US 2001-346117P 20011019.

AB Proteins consisting of two PDGF-D polypeptide chains, polynucleotides encoding the polypeptides, and materials and method for making the proteins are disclosed. Each of the polypeptide chains consists of, from amino terminus to carboxyl terminus, the following operably linked segments: P1-P2-h-CH2-CH3; P1-P2-CH2-CH3; h-CH2-CH3-P2-P1; or CH2-CH3-P2-P1. Within these polypeptide chains, P1 is a first polypeptide segment as shown in SEQ ID NO:2 or SEQ ID NO:4 from amino acid x to amino acid y, wherein x is an integer from 246 to 258, inclusive, and y is an integer from 365-370, inclusive; P2 is a second polypeptide segment consisting of from 4 to 20 amino acid residues; h is an Ig hinge region or portion thereof; and CH2 and CH3 are CH2 and CH3 domains of an Ig heavy chain, resp. Within the protein, the two polypeptide chains are joined by one or more disulfide bonds, each of the chain is optionally glycosylated, and the protein binds to and activates cell-surface PDGF receptors.

L12 ANSWER 14 OF 25 CAPLUS COPYRIGHT 2007 ACS on STN

2003:930854 Document No. 140:1645 Cloning and characterization of a new family of human secreted proteins - SCUBE1, SCUBE2 and SCUBE3 differentially expressed in vascular endothelium, and therapeutic use thereof. Yang, Ruey-bing; Ng, Chi Kin Domingos; Tomlinson, James E.; Komuves, Laszlo G.; Topper, James N.; Robison, Keith E. (Millennium Pharmaceuticals, Inc., USA). U.S. Pat. Appl. Publ. US 2003219813 A1 20031127, 86 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-406073 20030403. PRIORITY: US 2002-369876P 20020405.

AB The invention relates to SCUBE mols. and generally to gene expression in vascular endothelial cells. The invention specifically relates to the discovery of a novel gene family containing the genes and proteins referred to herein as SCUBE1, SCUBE2 and SCUBE3 which can be expressed in endothelial cells. SCUBE1, SCUBE2 and SCUBE3 genes are found to reside on human chromosome 22q13 and 11p15, and 6p21 resp. SCUBE genes encode secreted proteins containing a secretory signal region, a chain of EGF-like domains, and a CUB domain, that can be differentially expressed in human endothelial cells compared to other human cell types. For an example, SCUBE1 contains signal peptide (amino acids 1-22), 10 EGF-like domains

(amino acids 37-72; 78-115; 121-156; 166-202; 206-241; 245-280; 286-321; 327-360; 366-401; and 737-773 resp.), a spacer region (amino acid 487-503) and a CUB domain (amino acids 798-907). For the characterization of SCUBE1 function, provided are recombinant proteins of two SCUBE1 deletion proteins, containing amino acids 26-789 (deleting the CUB domain) and 26-411 (deleting the spacer, the 10th EGF-like domain and the CUB domain) resp. are prepared The spacer region is critical for the secretion and surface expression of SCUBE1. SCUBE1 is also a glycosylated membrane associated protein with homomeric interaction. Down-regulation of SCUBE1 and SCUBE2 expression by cellular signaling factors (IL-1β, TNF-α) or by Toxins (LPS) are demonstrated in monkey tissues. Furthermore, SCUBE1 is shown to interact with growth factors PDGF-C or PDGF-D . SCUBE proteins may be involved in the development of cardiovascular disease, hemostasis, thrombosis, inflammatory disease, bone metabolism disorders, urinary bladder disorders and breast disorders. The expression profiles for SCUBE1, SCUBE2 and SCUBE3 in human, mouse and monkey are provided; and SCUBE1 is detected in thrombi in many monkey tissues, including in kidney and spleen.

L12 ANSWER 15 OF 25 CAPLUS COPYRIGHT 2007 ACS on STN

2003:892428 Document No. 139:359243 Compositions and methods for modulating vasculogenesis or angiogenesis with platelet-derived growth factor C (PDGF-C) core protein domain. Li, Xuri; Eriksson, Ulf; Carmeliet, Peter; Collen, Desire (Ludwig Institute for Cancer Research, USA). U.S. Pat. Appl. Publ. US 2003211994 A1 20031113, 82 pp., Cont.-in-part of U.S. Ser. No. 410,349. (English). CODEN: USXXCO. APPLICATION: US 2002-303997 20021126. PRIORITY: US 1998-102461P 19980930; US 1998-108109P 19981112; US 1998-110749P 19981203; US 1998-113002P 19981218; US 1999-135426P 19990521; US 1999-144022P 19990715; US 1999-410349 19990930.

AB A method for modulating vasculogenesis or angiogenesis using the core domain protein of PDGF-C, a new member of the PDGF/VEGF family of growth factors, or a homodimer or a heterodimer comprising the core domain. Also disclosed are pharmaceutical compns. comprising the core protein, nucleotide sequences encoding the protein, and uses thereof in medical and diagnostic applications.

L12 ANSWER 16 OF 25 CAPLUS COPYRIGHT 2007 ACS on STN

2003:300603 Document No. 138:326530 Method for stimulating connective tissue growth or wound healing. Uutela, Marko; Eriksson, Ulf; Alitalo, Kari (Finland). U.S. Pat. Appl. Publ. US 2003073637 A1 20030417, 47 pp., Cont.-in-part of U.S. Ser. No. 86,623. (English). CODEN: USXXCO. APPLICATION: US 2002-260539 20021001. PRIORITY: US 1998-107852P 19981110; US 1998-113997P 19981228; US 1999-150604P 19990826; US 1999-157108P 19991004; US 1999-157756P 19991005; US 1999-438046 19991110; US 2000-691200 20001019; US 2002-86623 20020304.

AB The invention features PDGF-D, a new member of the PDGF/VEGF family of growth factors, as well as the nucleotide sequence encoding it, methods for producing it, antibodies and other antagonists to it, transfected and transformed host cells expressing it, pharmaceutical compns. containing it, and uses thereof in medical and diagnostic applications, including methods for stimulating growth of a connective tissue or healing a wound in a mammal, which methods comprise administering to the mammal an effective amount of PDGF-D polypeptides or polynucleotides encoding the PDGF-D polypeptides.

L12 ANSWER 17 OF 25 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2003:834994 The Genuine Article (R) Number: 725JR. Obstructive uropathy in mice and humans: Potential role for PDGF-D in the progression of tubulointerstitial injury. Taneda S; Hudkins K L; Topouzis S; Gilbertson D G; Ophascharoensuk V; Troung L; Johnson R J; Alpers C E (Reprint). Univ Washington, Med Ctr, Dept Pathol, Box 356100, 1959 NE Pacific St, Seattle, WA 98195 USA (Reprint); Univ Washington, Med Ctr, Dept Pathol, Seattle, WA 98195 USA; Univ Washington, Div Nephrol, Seattle,

WA 98195 USA; Zymogenet Inc, Seattle, WA 98105 USA; Baylor Coll Med, Dept Pathol, Houston, TX 77030 USA; Baylor Coll Med, Div Nephrol, Houston, TX 77030 USA. JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY (OCT 2003) Vol. 14, No. 10, pp. 2544-2555. ISSN: 1046-6673. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Tubulointerstitial fibrosis is a major characteristic of progressive renal diseases. Platelet-derived growth factor (PDGF) is a family of growth regulatory molecules consisting of PDGF-A and -B, along with the newly discovered PDGF-C and -D. They signal through cell membrane receptors, PDGF receptor alpha (PDGF-Ralpha) and receptor beta (PDGF-Rbeta). Involvement of PDGF-B and PDGF-Rbeta in the initiation and progression of renal fibrosis has been well documented. The authors studied the localization of PDGF ligands and receptors by immunchistochemistry, with emphasis on the role of PDGF-D in murine renal fibrosis induced by unilateral ureteral obstruction (UUO). In mice with UUO, de novo expression of PDGF-D was detected in interstitial cells at day 4, which increased to maximal expression at day 14. Increased expression of PDGF-B by interstitial cells and in some tubules was observed after day 4. The diseased mice did not show augmentation of PDGF-A or PDGF-C proteins in the areas of fibrosis. PDGF-Ralpha and -Rbeta protein expression was increased in interstitial cells after day 4 and reached maximal expression at day 14. Human renal nephrectornies (n = 10) of chronic obstructive nephropathy demonstrated similar de novo expression of PDGF-D in interstitial cells, correlating with expression of PDGF-Rbeta and PDGF-B, as it did in the murine model. These observations suggest that PDGF-D plays an important role in the pathogenesis of tubulointerstitial injury through binding of PDGF-Rbeta in both human obstructive nephropathy and the corresponding murine model of UUO.

L12 ANSWER 18 OF 25 MEDLINE on STN DUPLICATE 4

2003398231. PubMed ID: 12937299. A fully human monoclonal antibody (CR002) identifies PDGF-D as a novel mediator of mesangioproliferative glomerulonephritis. Ostendorf Tammo; van Roeyen Claudia R C; Peterson Jeffrey D; Kunter Uta; Eitner Frank; Hamad Avin J; Chan Gerlinde; Jia Xiao-Chi; Macaluso Jennifer; Gazit-Bornstein Gadi; Keyt Bruce A; Lichenstein Henri S; LaRochelle William J; Floege Jurgen. (Division Nephrology, University of Aachen, Germany.) Journal of the American Society of Nephrology : JASN, (2003 Sep) Vol. 14, No. 9, pp. 2237-47. Journal code: 9013836. ISSN: 1046-6673. Pub. country: United States. Language: English.

AB PDGF-B is of central importance in mesangioproliferative diseases. PDGF-D, a new PDGF isoform, like PDGF-B, signals through the PDGF betabeta-receptor. The present study first determined that PDGF-D is mitogenic for rat mesangial cells and is not inhibited by a PDGF-B antagonist. Low levels of PDGF-D mRNA were detected in normal rat glomeruli. After induction of mesangioproliferative nephritis in rats by anti-Thy 1.1 mAb, glomerular PDGF-D mRNA and protein expression increased significantly from days 4 to 9 in comparison with nonnephritic rats. Peak expression of PDGF-D mRNA occurred 2 d later than peak PDGF-B mRNA expression. In addition, PDGF-D serum levels increased significantly in the nephritic animals on day 7. For investigating the functional role of PDGF-D, neutralizing fully human mAb were generated using the XenoMouse technology. Rats with anti-Thy 1.1-induced nephritis were treated on days 3 and 5 with different amounts of a fully human PDGF-DD-specific neutralizing mAb (CR002), equal amounts of irrelevant control mAb, or PBS by intraperitoneal injection. Specific antagonism of PDGF-D led to a dose-dependent (up to 67%) reduction of glomerular cell proliferation. As judged by double immunostaining for 5-bromo-2'-deoxyuridine and alpha-smooth muscle actin, glomerular

mesangial cell proliferation was reduced by up to 57%. Reduction of glomerular cell proliferation in the rats that received CR002 was not associated with reduced glomerular expression of PDGF-B mRNA. PDGF-D antagonism also led to reduced glomerular infiltration of monocytes/macrophages (day 5) and reduced accumulation of fibronectin (day 8). In contrast, no effect was noted in normal rats that received an injection of CR002. These data show that PDGF-D is overexpressed in mesangioproliferative states and can act as an auto-, para-, or even endocrine glomerular cell mitogen, indicating that antagonism of PDGF-D may represent a novel therapeutic approach to mesangioproliferative glomerulonephritides.

L12 ANSWER 19 OF 25 CAPLUS COPYRIGHT 2007 ACS on STN

2002:850240 Document No. 137:363702 Platelet-derived growth factor D, DNA coding for it, and pharmaceutical uses. Eriksson, Ulf; Aase, Karin; Li, Xuri; Ponten, Annica; Uutela, Marko; Alitalo, Kari; Oestman, Arne; Heldin, Carl-Henrik (Ludwig Institute for Cancer Research, USA; Licentia Ltd.). U.S. Pat. Appl. Publ. US 2002164710 A1 20021107, 60 pp., Cont.-in-part of U. S. Ser. No. 691,200, abandoned. (English). CODEN: USXXCO. APPLICATION: US 2002-86623 20020304. PRIORITY: US 2000-691200 20001019; US 1999-438046 19991110; US 1998-107852P 19981110; US 1998-113997P 19981228; US 1999-150604P 19990826; US 1999-157108P 19991004; US 1999-157756P 19991005.

AB PDGF-D, a new member of the PDGF/VEGF family of polypeptide growth factors, is described, as well as nucleotide sequences encoding, methods for producing, pharmaceutical compns. containing this new growth factor, and its antibodies and other antagonists. Also disclosed are transfected and transformed host cells expressing PDGF-D, and uses thereof in medical and diagnostic applications. Fragments and homologs of PDGF-D are also covered by the invention.

L12 ANSWER 20 OF 25 CAPLUS COPYRIGHT 2007 ACS on STN

2002:540192 Document No. 137:104171 PDGF D polypeptides, nucleic acids encoding them, and therapeutic or diagnostic applications of the polypeptides or their antibodies. Shimkets, Richard A.; Lichenstein, Henri; Herrmann, John L.; Boldog, Ferenc L.; Minskoff, Stacey; Jeffers, Michael; Andrews, David; La Rochelle, William (USA). U.S. Pat. Appl. Publ. US 2002094546 A1 20020718, 97 pp., Cont.-in-part of U.S. Ser. No. 715,332. (English). CODEN: USXXCO. APPLICATION: US 2001-775482 20010202. PRIORITY: US 1999-158083P 19991007; US 1999-159231P 19991013; US 2000-174485P 20000104; US 2000-186707P 20000303; US 2000-188250P 20000310; US 2000-223879P 20000808; US 2000-234082P 20000920; US 2000-688312 20001013; US 2000-715332 20001116.

AB Disclosed are novel PDGFD nucleic acids encoding proteins and polypeptides related to bone morphogenetic protein-1 (BMF1), to vascular endothelial growth factor E (VEGF-E) and to platelet derived growth factor (PDGF). Also disclosed are vectors, host cells, antibodies, and recombinant methods for producing these nucleic acids and polypeptides. Methods of use include detecting and staging of cancers. The claims of this continuation-in-part patent specifically claim a method of detecting the presence of at least one PDGFD antigen in a sample, comprising the steps of: (a) providing a biol. sample; (b) contacting the sample with an agent that binds the antigen; and (c) detecting the presence of the agent bound to the antigen; whereby the presence of the agent indicates that the antigen is present in the sample. A method contributing to a diagnosis of cancer in a subject based on the presence of a PDGFD antigen in a sample from the subject is also claimed, as is a method of staging cancer in a subject. Addnl. claimed are a method of phosphorylating a tyrosine residue of a cellular receptor comprising the step of contacting a cell harboring the receptor with a PDGFD polypeptide, a method of stimulating a response in a cell that is specific for a PDGF beta receptor comprising contacting the cell with a PDGFD polypeptide, and a method of inhibiting the growth of a cell by contacting the cell with an agent that specifically binds a PDGFD polypeptide. An isolated nucleic acid

comprising a sequence encoding a PDGFD polypeptide and a method of preparing the PDGFD polypeptide are also claimed.

L12 ANSWER 21 OF 25 MEDLINE on STN DUPLICATE 5
2002242895. PubMed ID: 11980634. Platelet-derived growth factor D: tumorigenicity in mice and dysregulated expression in human cancer. LaRochelle William J; Jeffers Michael; Corvalan Jose R F; Jia Xiao-Chi; Feng Xiao; Vanegas Sandra; Vickroy Justin D; Yang Xiao-Dong; Chen Francine; Gazit Gadi; Mayotte Jane; Macaluso Jennifer; Rittman Beth; Wu Frank; Dhanabal Mohan; Herrmann John; Lichenstein Henri S. (CuraGen Corp., Branford, Connecticut 06405, USA.. wlarochelle@curagen.com) . Cancer research, (2002 May 1) Vol. 62, No. 9, pp. 2468-73. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.
AB Platelet-derived growth factor (PDGF) has been directly implicated in developmental and physiological processes, as well as in human cancer and other proliferative disorders. We have recently isolated and characterized a novel protease-activated member of the PDGF family, PDGF D. PDGF D has been shown to be proliferative for cells of mesenchymal origin, signaling through PDGF receptors. Comprehensive and systematic PDGF D transcript analysis revealed expression in many cell lines derived from ovarian, renal, and lung cancers, as well as from astrocytomas and medulloblastomas. beta PDGF receptor profiling further suggested autocrine signaling in several brain tumor cell lines. PDGF D transforming ability and tumor formation in SCID mice was further demonstrated. Exploiting a sensitive PDGF D sandwich ELISA using fully human monoclonal antibodies, PDGF D was detected at elevated levels in the sera of ovarian, renal, lung, and brain cancer patients. Immunohistochemical analysis confirmed PDGF D localization to ovarian and lung tumor tissues. Together, these data demonstrate that PDGF D plays a role in certain human cancers.

L12 ANSWER 22 OF 25 MEDLINE on STN DUPLICATE 6
2002667552. PubMed ID: 12427128. Platelet-derived growth factor-D expression in developing and mature human kidneys. Changsirikulchai Siribha; Hudkins Kelly L; Goodpaster Tracy A; Volpone John; Topouzis Stavros; Gilbertson Debra G; Alpers Charles E. (Department of Medicine, Srinakharinwirot University, Bangkok, Thailand.) Kidney international, (2002 Dec) Vol. 62, No. 6, pp. 2043-54. Journal code: 0323470. ISSN: 0085-2538. Pub. country: United States. Language: English.
AB BACKGROUND: Platelet-derived growth factor (PDGF) is a family of growth regulatory molecules composed of sulfide-bonded dimeric structures. Two well-studied PDGF peptides (PDGF-A and PDGF-B) have been shown to mediate a wide range of biological effects. PDGF-D is a newly recognized member of the PDGF family. Initial studies of the PDGF -D gene found its expression in cells of the vascular wall, suggesting that it could participate in vascular development and pathology. However, its localization in human kidney tissues has never been studied. METHODS: PDGF-D expression in fetal (N = 30) and adult (N = 25) human kidney tissues was examined by immunohistochemistry using an affinity-purified antibody raised to human PDGF-D. Antibody absorption with the immunizing peptide was employed to confirm the specificity of this antibody. PDGF-D protein and gene expression in human kidneys also were demonstrated by Western blotting and reverse transcription-polymerase chain reaction (RT-PCR). RESULTS: In the developing kidney, PDGF-D was first expressed by epithelial cells of comma- and S-shaped structures of the developing nephron, and most consistently in the visceral epithelial cells in the later stages of glomerular differentiation. In addition, PDGF-D could be found in mesenchymal, presumptively fibroblast cells in the interstitium of developing renal pelvis and in fetal smooth muscle cells in arterial vessels. In the adult normal kidney, PDGF-D was expressed by the visceral epithelial cells. There was

persistent expression in arterial smooth muscle cells as well as in some neointimal smooth muscle cells of arteriosclerotic vessels, and expression in smooth muscle cells of vasa rectae in the medulla. PDGF-D could be identified at the basolateral membrane of some injured tubules in areas of chronic tubulointerstitial injury routinely encountered in aging kidneys. Western blotting of homogenates of adult kidneys demonstrated monospecific bands at 50 kD corresponding to previously established size parameter for this protein. RT-PCR of human kidney RNA resulted in a 918 basepair band, the sequence of which corresponded to human PDGF-D (Genbank number AF336376). CONCLUSIONS: To our knowledge, these are the first studies to localize PDGF-D in human kidneys and suggest that PDGF-D may have a role in kidney development. PDGF-D was shown to bind to PDGF beta receptor, which localizes to mesangial cells, parietal epithelial cells, and interstitial fibroblasts, suggesting potential paracrine interactions between those cells and the visceral epithelium.

L12 ANSWER 23 OF 25 CAPLUS COPYRIGHT 2007 ACS on STN

2002:231900 Document No. 137:227182 Mice with Cre recombinase activatable PDGF-C expression. Ding, Hao; Wu, Xiaoli; Nagy, Andras (Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, M5G 1X5, Can.). Genesis (New York, NY, United States), 32(2), 181-183 (English) 2002. CODEN: GNESFY. ISSN: 1526-954X. Publisher: Wiley-Liss, Inc..

AB The recent discovery of two new platelet-derived growth factor (PDGF) family members, PDGF-C and PDGF-D, suggests that the functional complexity of the PDGF family is larger than previously thought. To analyze the consequences of ectopic or overexpression of PDGF-C, the authors present the establishment of conditional transgenic mice that express PDGF-C in a Cre-excision conditional manner. Western blot anal. with anti-Flag antibody showed two PDGF-C isoforms in double transgenic embryos, i.e., the full-length 55 kDa and the protease-activated 23 kDa isoform. Preliminary observation of midgestation double transgenic embryos indicated that biol. activity of transgenic PDGF-C caused developmental defects, including facial abnormalities.

L12 ANSWER 24 OF 25 CAPLUS COPYRIGHT 2007 ACS on STN

2000:535282 Document No. 133:145921 Human platelet-derived growth factor/vascular endothelial growth factor-like growth factor H, its cDNA sequences and therapeutic applications. Eriksson, Ulf; Alitalo, Kari; Lauren, Juha (Ludwig Institute for Cancer Research, USA; Helsinki University Licensing Ltd.). PCT Int. Appl. WO 2000044903 A1 20000803, 59 pp. DESIGNATED STATES: W: AU, CA, CN, JP, KR, NZ, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US1895 20000128. PRIORITY: US 1999-PV117864 19990129.

AB A portion of the PDGF/VEGF-Like Growth Factor H, a new member of the VEGF family of growth factors, is described, as well as its cDNA sequences. Methods for expressing and producing it, analyzing its function, preparing its antibodies and screening for its antagonists for medical and diagnostic applications are also provided.

L12 ANSWER 25 OF 25 CAPLUS COPYRIGHT 2007 ACS on STN

2000:335442 Document No. 133:1492 Human platelet-derived growth factor D, its cDNA sequences, and uses thereof in medical and diagnostic applications. Eriksson, Ulf; Aase, Karin; Ponten, Annica; Lee, Xuri; Uutela, Marko; Alitalo, Kari; Oestman, Arne; Heldin, Carl-Henrik (Ludwig Institute for Cancer Research, USA; Helsinki University Licensing Ltd. Oy (FI/FI)). PCT Int. Appl. WO 2000027879 A1 20000518, 111 pp. DESIGNATED STATES: W: AE, AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GD, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.

(English). CODEN: PIXXD2. APPLICATION: WO 1999-US26462 19991110. PRIORITY: US 1998-PV107852 19981110; US 1998-PV113997 19981228; US 1999-PV150604 19990826; US 1999-PV157108 19991004; US 1999-PV157756 19991005.

AB PDGF-D, a new member of the PDGF/VEGF family of growth factors, is described, as well as the nucleotide sequence encoding it, methods for producing it, antibodies and other antagonists to it, transfected and transformed host cells expressing it, pharmaceutical compns. containing it, and uses thereof in medical and diagnostic applications.

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L15 4 DUP REMOVE L14 (12 DUPLICATES REMOVED)

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L15 ANSWER 1 OF 4 MEDLINE on STN DUPLICATE 1

2006205861. PubMed ID: 16557224. Biological responses to PDGF-BB versus PDGF-DD in human mesangial cells. van Roeyen C R C; Ostendorf T; Denecke B; Bokemeyer D; Behrmann I; Strutz F; Lichenstein H S; LaRochelle W J; Pena C E; Chaudhuri A; Floege J. (Division of Nephrology, RWTH Aachen University, Aachen, Germany.) Kidney international, (2006 Apr) Vol. 69, No. 8, pp. 1393-402. Journal code: 0323470. ISSN: 0085-2538. Pub. country: United States. Language: English.

AB Platelet-derived growth factor (PDGF)-BB and PDGF-DD mediate mesangial cell proliferation in vitro and in vivo. While PDGF-BB is a ligand for the PDGF alpha- and beta-receptor chains, PDGF-DD binds more selectively to the beta-chain, suggesting potential differences in the biological activities. Signal transduction and regulation of gene expression induced by PDGF-BB and -DD were compared in primary human mesangial cells (HMCs), which expressed PDGF alpha- and beta-receptor subunits. The growth factor concentrations used were chosen based on their equipotency in inducing HMCs proliferation and binding to the betabeta-receptor. Both growth factors, albeit at different concentrations induced phosphorylation and activation of extracellular signal-regulated kinase 1 (ERK1) and ERK2. In addition, PDGFs led to the phosphorylation and activation of signal transducers and activators of transcription 1 (STAT1) and STAT3. HMCs proliferation induced by either PDGF-BB or -DD could be blocked by signal transduction inhibitors of the mitogen-activated protein kinase-, Janus kinase (JAK)/STAT-, or phosphatidyl-inositol 3-kinase pathways. Using a gene chip array and subsequent verification by real-time reverse transcriptase (RT)-polymerase chain reaction, we found that in HMC genes for matrix metalloproteinase 13 (MMP-13) and MMP-14 and, to a low extent, cytochrome B5 and cathepsin L were exclusively regulated by PDGF-BB, whereas no exclusive gene regulation was detected by PDGF-DD. However, at the protein level, both MMP-13 and -14 were equally induced by PDGF-BB and -DD. PDGF-BB and -DD effect similar biological responses in HMCs albeit at different potencies. Rare apparently differential gene regulation did not result in different protein expression, suggesting that in HMCs both PDGFs exert their biological activity almost exclusively via the PDGF beta-receptor.

L15 ANSWER 2 OF 4 MEDLINE on STN DUPLICATE 2

2005445381. PubMed ID: 16039137. Expression patterns of PDGF-A, -B, -C and -D and the PDGF-receptors alpha and beta in activated rat hepatic stellate cells (HSC). Breitkopf Katja; Roeyen Claudia van; Sawitza Iris; Wickert Lucia; Floege Jurgen; Gressner Axel M. (Department of Medicine II, Mol. Alcohol Research in Gastroenterology, University Hospital Mannheim, University of Heidelberg, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany.. katja.breitkopf@med.ma.uni-heidelberg.de) . Cytokine, (2005 Sep 7) Vol. 31, No. 5, pp. 349-57. Journal code: 9005353. ISSN: 1043-4666. Pub. country: United States. Language: English.

AB The platelet-derived growth factor (PDGF) family, which regulates many physiological and pathophysiological processes has recently been enlarged by two new members, the isoforms PDGF-C and -D. Little is known about the expression levels of these new members in hepatic fibrosis. We therefore investigated by quantitative real time PCR (Taqman) the mRNA expression profiles of all four PDGF isoforms in transdifferentiating primary cultured hepatic stellate cells (HSC), an in vitro model system of hepatic fibrogenesis, either with or without stimulation of the cells with PDGF-BB or TGF-beta1. All four isoforms were expressed in HSC transdifferentiating to myofibroblast-like cells (MFB) albeit with different profiles: while PDGF-A mRNA exhibited minor fluctuations only, PDGF-B was rapidly down-regulated. In contrast, both PDGF-C and -D mRNA were strongly induced: PDGF-C up to 5 fold from day 2 to day 8 and PDGF-D up to 8 fold from day 2 to day 5 of culture. Presence of PDGF-DD in activated HSC was confirmed at the protein level by immunocytochemistry. Stimulation of HSC and MFB with PDGF-BB led to down-regulation of the new isoforms, whereas TGF-beta1 upregulated PDGF-A only. We further show that PDGF receptor-beta (PDGFR-beta) mRNA was rapidly upregulated within the first day of culture and was constantly expressed from day 2 on while the expression profile of PDGFR-alpha mRNA was very similar to that of PDGF-A during transdifferentiation. Given the dramatic changes in PDGF-C and -D expression, which may compensate for down-regulation of PDGF-B, we hypothesize that the new PDGF isoforms may fulfil specific functions in hepatic fibrogenesis.

L15 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2007 ACS on STN

2004:252313 Document No. 140:286157 Anti-PDGF-DD antibodies for diagnosis and treatment of nephritis and related diseases. Floege, Juergen; Gazit-Bornstein, Gadi; Keyt, Bruce; Larochelle, William J.; Lichenstein, Henri (Abgenix, Inc., USA; Curagen Corporation). PCT Int. Appl. WO 2004024098 A2 20040325, 115 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US29414 20030916. PRIORITY: US 2002-411137P 20020916.

AB Embodiments of the invention described herein relate to antibodies directed to platelet derived growth factor-DD (PDGF-DD) and uses of such antibodies. The antibodies of the invention find use as diagnostics and as treatments for diseases associated with the overprodn. of PDGF-DD. In particular, in accordance with embodiments of the invention, the use of anti-PDGF-DD antibodies for the treatment of nephritis and related disorders, including diseases caused by mesangial proliferation is provided.

L15 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 3

2003398231. PubMed ID: 12937299. A fully human monoclonal antibody (CR002) identifies PDGF-D as a novel mediator of mesangioproliferative glomerulonephritis. Ostendorf Tammo; van Roeyen Claudia R C; Peterson Jeffrey D; Kunter Uta; Eitner Frank; Hamad Avin J; Chan Gerlinde; Jia Xiao-Chi; Macaluso Jennifer; Gazit-Bornstein Gadi; Keyt

Bruce A; Lichenstein Henri S; LaRochelle William J ; Floege Jurgen. (Division Nephrology, University of Aachen, Germany.) Journal of the American Society of Nephrology : JASN, (2003 Sep) Vol. 14, No. 9, pp. 2237-47. Journal code: 9013836. ISSN: 1046-6673. Pub. country: United States. Language: English.

AB PDGF-B is of central importance in mesangioproliferative diseases. PDGF-D, a new PDGF isoform, like PDGF-B, signals through the PDGF betabeta-receptor. The present study first determined that PDGF-D is mitogenic for rat mesangial cells and is not inhibited by a PDGF-B antagonist. Low levels of PDGF-D mRNA were detected in normal rat glomeruli. After induction of mesangioproliferative nephritis in rats by anti-Thy 1.1 mAb, glomerular PDGF-D mRNA and protein expression increased significantly from days 4 to 9 in comparison with nonnephritic rats. Peak expression of PDGF-D mRNA occurred 2 d later than peak PDGF-B mRNA expression. In addition, PDGF-D serum levels increased significantly in the nephritic animals on day 7. For investigating the functional role of PDGF-D, neutralizing fully human mAb were generated using the XenoMouse technology. Rats with anti-Thy 1.1-induced nephritis were treated on days 3 and 5 with different amounts of a fully human PDGF-DD -specific neutralizing mAb (CR002), equal amounts of irrelevant control mAb, or PBS by intraperitoneal injection. Specific antagonism of PDGF-D led to a dose-dependent (up to 67%) reduction of glomerular cell proliferation. As judged by double immunostaining for 5-bromo-2'-deoxyuridine and alpha-smooth muscle actin, glomerular mesangial cell proliferation was reduced by up to 57%. Reduction of glomerular cell proliferation in the rats that received CR002 was not associated with reduced glomerular expression of PDGF-B mRNA. PDGF-D antagonism also led to reduced glomerular infiltration of monocytes/macrophages (day 5) and reduced accumulation of fibronectin (day 8). In contrast, no effect was noted in normal rats that received an injection of CR002. These data show that PDGF-D is overexpressed in mesangioproliferative states and can act as an auto-, para-, or even endocrine glomerular cell mitogen, indicating that antagonism of PDGF-D may represent a novel therapeutic approach to mesangioproliferative glomerulonephritides.

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L3          635 S L2 AND NEPHRITIS
L4          288 S L3 AND ANTIBOD?
L5            0 S L4 AND PDGF-D
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L9           46 S L8 AND PDGF-D
L10           6 S L9 AND PDGF-DD
L11           2 DUP REMOVE L10 (4 DUPLICATES REMOVED)
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L18 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN
2006:1174166 Document No. 145:487670 Humanized anti-interleukin-6 antibodies for immunotherapy. Chen, Yan; Gardner, Debra; Knight, David M.; Lark, Michael W.; Liang, Bailin; Shealy, David J.; Song, Xiao-Yu R.; Stojanovic-Susulic, Vedrana; Sweet, Raymond W.; Tam, Susan H.; Wu, Sheng-Jiun; Yang, Jing; Marquis, David Matthew; Smith, Eric Michael; Vasserot, Alain Philippe (Centocor, Inc., USA; Applied Molecular Evolution, Inc.). PCT Int. Appl. WO 2006119115 A2 20061109, 229pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2006-US16457 20060428. PRIORITY: US 2005-676498P 20050429; US 2005-677319P 20050503.
AB The authors disclose the engineering and biol. activity of mouse CDR-engrafted human antibodies with specificity for interleukin-6.

L18 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN
2005:1170699 Document No. 143:438500 Human monoclonal anti-human CD20 antibodies and derivatives for diagnosis and treatment of CD20-associated neoplastic, inflammatory and autoimmune diseases. Teeling, Jessica; Glennie, Martin; Parren, Paul; Gerritsen, Arnout F.; Ruuls, Sigrid; Graus, Yvo; Van De Winkel, Jan (Genmab A/S, Den.). PCT Int. Appl. WO 2005103081 A2 20051103, 106 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-DK270 20050420. PRIORITY: DK 2004-627 20040420.
AB Isolated human monoclonal antibodies which bind to and inhibit human CD20, and related antibody-based compns. and mols., are disclosed. Also disclosed are pharmaceutical compns. comprising the human antibodies, and therapeutic and diagnostic methods for using the human antibodies.

L18 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN
2004:354963 Document No. 140:373902 Transgenic animal-produced human monoclonal antibodies against human CD20 for diagnosis and treatment of cancer, immune disease, inflammation and autoimmune disease. Teeling, Jessica; Ruuls, Sigrid; Glennie, Martin; Van De Winkel, Jan G. J.; Parren, Paul; Petersen, Jorgen; Baadsgaard, Ole; Huang, Haichun (Genmab A/S, Den.; Medarex, Inc.). PCT Int. Appl. WO 2004035607 A2 20040429, 201 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2.

APPLICATION: WO 2003-US33057 20031017. PRIORITY: US 2002-419163P 20021017; US 2003-460028P 20030402.

AB Isolated human monoclonal antibodies which bind to and inhibit human CD20, and related antibody-based compns. and mols., are disclosed. The human antibodies can be produced by a transfectoma or in a non-human transgenic animal, e.g., a transgenic mouse, capable of producing multiple isotypes of human monoclonal antibodies by undergoing V-D-J recombination and isotype switching. Also disclosed are pharmaceutical compns. comprising the human antibodies, non-human transgenic animals and hybridomas which produce the human antibodies, and therapeutic and diagnostic methods for using the human antibodies.

=> s inhibit
L19 1288173 INHIBIT

=> s l19 and mesangial cell proliferation
L20 683 L19 AND MESANGIAL CELL PROLIFERATION

=> s l20 and anti-PDGF
L21 5 L20 AND ANTI-PDGF

=> dup remove l21
PROCESSING COMPLETED FOR L21
L22 1 DUP REMOVE L21 (4 DUPLICATES REMOVED)

=> d l22 cbib abs

L22 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1
2002632330. PubMed ID: 12390311. IL-10 induces mesangial cell proliferation via a PDGF-dependent mechanism.
Robertson T E; Nikolic-Paterson D J; Hurst L A; Atkins R C; Chadban S J.
(Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.) Clinical and experimental immunology, (2002 Nov) Vol. 130, No. 2, pp. 241-4. Journal code: 0057202. ISSN: 0009-9104. Pub. country: England: United Kingdom. Language: English.

AB Interleukin-10 (IL-10) is a mesangial cell growth factor in vivo and in vitro. However, the mechanism by which IL-10 exerts its mitogenic activity is not known. The aim of this study was to determine whether IL-10 induces mesangial cell proliferation in a PDGF-dependent or independent fashion. A well--characterized rat mesangial cell line (1097) was used in a series of cell proliferation experiments in which cells were serum-starved and then incubated with recombinant IL-10 in the presence or absence of STI 571 (a specific inhibitor of signalling via the PDGF-alpha and beta receptors) or a neutralizing anti-PDGF-AB antibody. IL-10 induced significant mesangial cell proliferation at 24 and 48 h after cytokine addition. This response was inhibited totally by the addition of STI-571, demonstrating that IL-10 mitogenic activity has an absolute requirement for signalling through the PDGF receptor. In further studies, it was found that STI-571 could be added 24 h after IL-10 stimulation and still exert a profound inhibition of IL-10 mitogenic activity. The ability of a neutralizing anti-PDGF-AB antibody to inhibit completely IL-10-induced mesangial cell proliferation confirmed that IL-10 acts via induction of an autocrine PDGF response rather than the possibility that IL-10 may transactivate the PDGF receptor in a PDGF-independent fashion. In conclusion, this study has demonstrated that IL-10 induces mesangial cell proliferation via an autocrine PDGF-mediated mechanism. Thus, therapies which antagonize PDGF signalling will also inhibit any contribution of IL-10 to mesangial proliferation.

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=> s l20 and antibod?
L23          87 L20 AND ANTIBOD?

=> s l23 and PDGF-DD
L24           0 L23 AND PDGF-DD

=> s l23 and PDGF-D
L25           0 L23 AND PDGF-D

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L26          23 L23 AND PDGF

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L27           13 DUP REMOVE L26 (10 DUPLICATES REMOVED)

=> d l27 1-13 cbib abs

L27  ANSWER 1 OF 13     MEDLINE on STN                    DUPLICATE 1
2005570711.    PubMed ID: 16248246.   Preventive effect of multi-glycoside of
     tripterygium Wilfordii Hook. f. on proteinuria and mesangial injury in
     experimental mesangial proliferative glomerulonephritis. Wan Yi-gang; Sun
     Wei; Zhen Yan-jun. (Department of Traditional Chinese Medicine, The
     Affiliated Drum Tower Hospital of Nanjing University Medical School,
     Nanjing. ) Zhongguo Zhong xi yi jie he za zhi Zhongguo Zhongxiyi jiehe
     zazhi = Chinese journal of integrated traditional and Western medicine /
     Zhongguo Zhong xi yi jie he xue hui, Zhongguo Zhong yi yan jiu yuan zhu
     ban, (2005 Sep) Vol. 25, No. 9, pp. 817-21.  Journal code: 9211576. ISSN:
     1003-5370. Pub. country: China. Language: Chinese.
AB   OBJECTIVE: To observe the preventive effect of multi-glycoside of
     Tripterygium Wilfordii Hook. f. (GYW) on proteinuria and mesentery injury
     in experimental mesangial proliferative glomerulonephritis in vivo.
     METHODS: The reversible anti-Thyl.1 antibody glomerulo nephritis
     model of rats was established with monoclonal antibody 1-22-3
     and intervened with GTW, and a control group was set up in the same time.
     Changes of 24h urinary protein excretion, serum creatinine (Scr), blood
     urea nitrogen (BUN), total plasma protein (TP) and glomerular morphology
     were observed, and the level of mRNA expression of proliferative factors,
     including platelet-derived growth factor-BB (PDGF-BB) and
     transforming growth factor-beta (TGF-beta), in renal tissue was
     determined.  RESULTS: GTW could inhibit proteinuria and
     mesangial injury in anti-Thyl. 1 antibody nephritis model.  The
     PDGF-BB and TGF-beta mRNA expression in the anti-Thy1.1
     antibody nephritis model rats were increased for 2.84 and 1.64
     times respectively to those in the normal control group.  GTW could
     down-regulate the over-expression of PDGF-BB mRNA by 33.1%, it
     was significantly different to that in the control group (P < 0.05).
     CONCLUSION: GTW could reduce the proteinuria and inhibit
     mesangial cells proliferation and
     extracellular matrix deposition, these effects maybe related to the
     down-regulating of PDGF-BB mRNA expression.

L27  ANSWER 2 OF 13  CAPLUS  COPYRIGHT 2007 ACS on STN
2003:503290   Document No. 139:255722  Counteractive effects of HGF on
     PDGF-induced mesangial cell
     proliferation in a rat model of glomerulonephritis.  Bessho,
     Kazuhiko; Mizuno, Shinya; Matsumoto, Kunio; Nakamura, Toshikazu (Division
     of Molecular Regenerative Medicine, Course of Advanced Medicine, Osaka
     University Graduate School of Medicine, Osaka, 565-0871, Japan).  American
     Journal of Physiology, 284(6, Pt. 2), F1171-F1180 (English) 2003.  CODEN:
     AJPHAP.  ISSN: 0002-9513.  Publisher: American Physiological Society.
AB   Activation and proliferation of glomerular mesangial cells play an
     important role in the development of mesangial proliferative
     glomerulonephritis.  The authors investigated the role of hepatocyte
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growth factor (HGF) in regulating activated mesangial cell proliferation. In glomeruli of normal rats, mesangial cells barely expressed the c-Met/HGF receptor. However, when mesangial proliferative glomerulonephritis was induced in rats by the administration of an anti-Thy 1.1 antibody, glomerular HGF expression transiently decreased along with mesangiolysis, and activation of mesangial cells was associated with upregulation of the c-Met receptor. Activated mesangial cells in culture also expressed the c-Met/HGF receptor. Although addition of HGF to cultured mesangial cells did not increase DNA synthesis, HGF did diminish PDGF-induced DNA synthesis. PDGF induced activation of ERK, which continued for at least 48 h. When PDGF and HGF were simultaneously added, HGF inhibited the prolonged activation of ERK, which suggests that early inactivation of PDGF-induced ERK may be involved in the inhibitory effect of HGF on mesangial cell proliferation. Furthermore, administration of HGF to rats with anti-Thy 1.1 nephritis resulted in a selective suppression of activated mesangial cell proliferation, and this suppressive effect was associated with attenuation of phosphorylated glomerular ERK. These results indicate that HGF counteracts PDGF -induced mesangial cell proliferation and functions as a neg. regulator of activated mesangial cell proliferation.

L27 ANSWER 3 OF 13 MEDLINE on STN DUPLICATE 2
2002632330. PubMed ID: 12390311. IL-10 induces mesangial cell proliferation via a PDGF-dependent mechanism. Robertson T E; Nikolic-Paterson D J; Hurst L A; Atkins R C; Chadban S J. (Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.) Clinical and experimental immunology, (2002 Nov) Vol. 130, No. 2, pp. 241-4. Journal code: 0057202. ISSN: 0009-9104. Pub. country: England: United Kingdom. Language: English.

AB Interleukin-10 (IL-10) is a mesangial cell growth factor in vivo and in vitro. However, the mechanism by which IL-10 exerts its mitogenic activity is not known. The aim of this study was to determine whether IL-10 induces mesangial cell proliferation in a PDGF-dependent or independent fashion. A well--characterized rat mesangial cell line (1097) was used in a series of cell proliferation experiments in which cells were serum-starved and then incubated with recombinant IL-10 in the presence or absence of STI 571 (a specific inhibitor of signalling via the PDGF-alpha and beta receptors) or a neutralizing anti-PDGF-AB antibody. IL-10 induced significant mesangial cell proliferation at 24 and 48 h after cytokine addition. This response was inhibited totally by the addition of STI-571, demonstrating that IL-10 mitogenic activity has an absolute requirement for signalling through the PDGF receptor. In further studies, it was found that STI-571 could be added 24 h after IL-10 stimulation and still exert a profound inhibition of IL-10 mitogenic activity. The ability of a neutralizing anti-PDGF-AB antibody to inhibit completely IL-10-induced mesangial cell proliferation confirmed that IL-10 acts via induction of an autocrine PDGF response rather than the possibility that IL-10 may transactivate the PDGF receptor in a PDGF -independent fashion. In conclusion, this study has demonstrated that IL-10 induces mesangial cell proliferation via an autocrine PDGF-mediated mechanism. Thus, therapies which antagonize PDGF signalling will also inhibit any contribution of IL-10 to mesangial proliferation.

L27 ANSWER 4 OF 13 CAPLUS COPYRIGHT 2007 ACS on STN
2001:301245 Document No. 135:286855 Gas6 regulates mesangial cell proliferation through Axl in experimental glomerulonephritis. Yanagita, Motoko; Arai, Hidenori; Ishii, Kenji; Nakano, Toru; Ohashi, Kazumasa; Mizuno, Kensaku; Varnum, Brian; Fukatsu,

Atsushi; Doi, Toshio; Kita, Toru (Department of Geriatric Medicine, Kyoto University, Kyoto, Japan). American Journal of Pathology, 158(4), 1423-1432 (English) 2001. CODEN: AJPAA4. ISSN: 0002-9440. Publisher: American Society for Investigative Pathology.

AB Proliferation of mesangial cells is a hallmark of glomerular disease, and understanding its regulatory mechanism is clin. important. Previously, the authors demonstrated that the product of growth arrest-specific gene 6 (Gas6) stimulates mesangial cell proliferation through binding to its cell-surface receptor Axl in vitro. We also showed that warfarin and the extra-cellular domain of Axl conjugated with Fc portion of human IgG1 (Axl-Fc) inhibit mesangial cell proliferation by interfering the Gas6/Axl pathway in vitro. In the present study, therefore, the authors examined in vivo roles of Gas6 and Axl in an exptl. model of mesangial proliferative glomerulonephritis induced by the injection of anti-Thy1.1 antibody (Thy1 GN). In Thy1 GN, expression of Gas6 and Axl was markedly increased in glomeruli, and paralleled the progression of mesangial cell proliferation. Administration of warfarin or daily injection of Axl-Fc inhibited mesangial cell proliferation, and abolished the induction of platelet-derived growth factor-B mRNA and protein in Thy1 GN. Moreover, the anti-proliferative effect of warfarin was achieved at lower concns. than those in routine clin. use. These findings indicate that the Gas6/Axl pathway plays a key role in mesangial cell proliferation in vivo, and could be a potentially important therapeutic target for the treatment of renal disease.

L27 ANSWER 5 OF 13 MEDLINE on STN DUPLICATE 3

1999341256. PubMed ID: 10412745. Effect of simvastatin on proliferative nephritis and cell-cycle protein expression. Yoshimura A; Nemoto T; Sugenoya Y; Inui K; Watanabe S; Inoue Y; Sharif S; Yokota N; Uda S; Morita H; Ideura T. (Department of Medicine, Showa University Fujigaoka Hospital, Yokohama, Japan.) Kidney international. Supplement, (1999 Jul) Vol. 71, pp. S84-7. Ref: 7. Journal code: 7508622. ISSN: 0098-6577. Pub. country: United States. Language: English.

AB BACKGROUND: Mesangial cell proliferation is important in subsequent mesangial matrix expansion in glomerular injury. Therefore, the regulation of mesangial cell proliferation may be critical in the treatment of glomerulonephritis. Inhibition of 3-hydro-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibits the production of mevalonate and has been shown to suppress proliferation in many cell types, including mesangial cells in vitro. It is expected that HMG-CoA reductase inhibitor may suppress mesangial cell proliferation and subsequent progression of glomerulonephritis. Recently, the tight relationship between cell-cycle regulatory protein expression and mesangial cell proliferation in experimental glomerulonephritis was demonstrated. The aim of the present study is to examine the effect of simvastatin, one of the HMG-CoA reductase inhibitors, on the glomerular cell proliferation and on the expression of CDK2 or p27Kip1 in mesangial cells in experimental glomerulonephritis in vivo. METHODS: The effect of simvastatin on a rat mesangial proliferative glomerulonephritis induced by antithymocyte antibody (anti-Thy 1.1 GN) was studied. Administration of simvastatin or vehicle (for control GN) were started from two days before disease induction, and was continued to the day of nephrectomy. Nephrectomy was done at days 0, 2, 4, 7, 12 and 20 after disease induction. Immunohistochemistry for proliferating cells, macrophages, alpha-smooth muscle actin, type IV collagen and PDGF-B chain was performed, respectively, in addition to conventional periodic-acid Schiff staining. Double immunostaining for CDK2/OX-7 or p27Kip1/OX-7 was also done, respectively. RESULTS: There was no difference in the degree of the initial injuries between simvastatin-treated and control GN rats. The most pronounced feature of simvastatin-treated GN was the suppression of the early glomerular cell proliferation (about 70% of proliferation was suppressed

at day 4). At day 4, alpha-smooth muscle actin expression was also decreased in simvastatin-treated GN rats. Inhibition of macrophage recruitment into glomeruli by simvastatin was also a prominent feature (about 30% decrease in the number of glomerular macrophages at day 2). Simvastatin significantly suppressed subsequent mesangial matrix expansion and type IV collagen accumulation in glomeruli. Although it might simply reflect the reduction in mesangial cells, glomerular PDGF-B chain expression was reduced. There was no significant difference in plasma lipids levels at day 2 and day 4. In vehicle-treated GN rats, the number of CDK2+/OX-7+ cells (CDK2-expressed mesangial cells) in glomeruli increased significantly from day 4 to day 7. Although simvastatin suppressed mesangial cell proliferation, the increase in the number of glomerular CDK2+/OX-7+ cells was also attenuated by simvastatin treatment. There was no difference in the number of p27Kip1+/OX-7+ cells (p27Kip1-expressed mesangial cells) in the glomerulus between vehicle-treated and simvastatin-treated GN rats. CONCLUSION: Simvastatin suppressed mesangial cell proliferation and subsequent matrix expansion, and macrophage infiltration into glomeruli in anti-Thy 1.1 GN rats. The antiproliferative effect of simvastatin in this model was also associated with the reduction of CDK2 expression in mesangial cells.

L27 ANSWER 6 OF 13 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1999:483481 The Genuine Article (R) Number: 209ZZ. Effect of simvastatin on proliferative nephritis and cell-cycle protein expression. Yoshimura A (Reprint); Nemoto T; Sugenoya Y; Inui K; Watanabe S; Inoue Y; Sharif S; Yokota N; Uda S; Morita H; Ideura T. Showa Univ, Fujigaoka Hosp, Dept Med, Div Nephrol, Aoba Ku, 1-30 Fujiaoka, Yokohama, Kanagawa 2278501, Japan (Reprint); Showa Univ, Fujigaoka Hosp, Dept Med, Div Nephrol, Aoba Ku, Yokohama, Kanagawa 2278501, Japan. KIDNEY INTERNATIONAL (JUL 1999) Vol. 56, Supp. [71], pp. S84-S87. ISSN: 0085-2538. Publisher: BLACKWELL SCIENCE INC, 350 MAIN ST, MALDEN, MA 02148 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background. mesangial cell proliferation is important in subsequent mesangial matrix expansion in glomerular injury. Therefore, the regulation of mesangial cell proliferation may be critical in the treatment of glomerulonephritis. Inhibition of 3-hydro-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibits the production of mevalonate and has been shown to suppress proliferation in many cell types, including mesangial cells in vitro. It is expected that HMG-CoA reductase inhibitor may suppress mesangial cell proliferation and subsequent progression of glomerulonephritis. Recently, the tight relationship between cell-cycle regulatory protein expression and mesangial cell proliferation in experimental glomerulonephritis was demonstrated, The aim of the present study is to examine the effect of simvastatin, one of the HMG-CoA reductase inhibitors, on the glomerular cell proliferation and on the expression of CDK2 or-p27Kip1 in mesangial cells in experimental glomerulonephritis in vivo.

Methods. The effect of simvastatin on a rat mesangial proliferative glomerulonephritis induced by antithymocyte antibody (anti-Thy 1.1 GN) was studied. Administration of simvastatin or vehicle (for control GN) were started from two days before disease induction, and was continued to the day of nephrectomy. Nephrectomy was done at days 0, 2, 4, 7, 12 and 20 after disease induction. Immunohistochemistry for proliferating cells. macrophages, or-smooth muscle actin, type IV collagen and PDGF-B chain was performed, respectively, in addition to conventional periodic-acid Schiff staining. Double immunostaining for CDK2/OX-7 or p27Kip1/OX-7 was also done, respectively.

Results. There was no difference in the degree of the initial injuries between simvastatin-treated and control GN rats. The most pronounced feature of simvastatin-treated GN was the suppression of the early glomerular cell proliferation (about 70% of proliferation was

suppressed at day 4). At day 4, alpha-smooth muscle actin expression was also decreased in simvastatin-treated GN rats. Inhibition of macrophage recruitment into glomeruli by simvastatin was also a prominent feature (about 30% decrease in the number of glomerular macrophages at day 2), Simvastatin significantly suppressed subsequent mesangial matrix expansion and type IV collagen accumulation in glomeruli. Although it might simply reflect the reduction in mesangial cells. glomerular PDGF-B chain expression was reduced. There was no significant difference in plasma lipids levels at day 2 and day 4. In vehicle-treated GN rats, the number of CDK2+/OX-7+ cells (CDK2-expressed mesangial cells) in glomeruli increased significantly from day 4 to day 7. Although simvastatin suppressed mesangial cell proliferation, the increase in the number of glomerular CDK2+/OX7+ cells was also attenuated by simvastatin treatment, There was no difference in the number of p27Kip1 +/OX-7+ cells (p27Kip1-expressed mesangial cells) in the glomerulus between vehicle-treated and simvastatin-treated GN rots.

Conclusion. Simvastatin suppressed mesangial cell proliferation and subsequent matrix expansion, and macrophage infiltration into glomeruli in anti-Thy 1.1 GN rats. The antiproliferative effect of simvastatin in this model was also associated with the reduction of CDK2 expression in mesangial cells.

L27 ANSWER 7 OF 13 EMBASE DUPLICATE 4

1999229512 EMBASE Effect of simvastatin on proliferative nephritis and cell-cycle protein expression. Yoshimura A.; Nemoto T.; Sugenoya Y.; Inui K.; Watanabe S.; Inoue Y.; Sharif S.; Yokota N.; Uda S.; Morita H.; Ideura T.. Dr. A. Yoshimura, Department of Medicine, Division of Nephrology, Showa University, 1-30 Fujigaoka, Aoba-ku, Yokohama 227-8501, Japan. Kidney International, Supplement Vol. 56, No. 71, pp. S84-S87 1999. Refs: 10.
ISSN: 0098-6577. CODEN: KISUDF
Pub. Country: United States. Language: English. Summary Language: English.
Entered STN: 19990715. Last Updated on STN: 19990715

AB Background. Mesangial cell proliferation is important in subsequent mesangial matrix expansion in glomerular injury. Therefore, the regulation of mesangial cell proliferation may be critical in the treatment of glomerulonephritis. Inhibition of 3-hydro-3-methylglutaryl coenzyme A (HMG- CoA) reductase inhibits the production of mevalonate and has been shown to suppress proliferation in many cell types, including mesangial cells in vitro. It is expected that HMG-CoA reductase inhibitor may suppress mesangial cell proliferation and subsequent progression of glomerulonephritis. Recently, the tight relationship between cell-cycle regulatory protein expression and mesangial cell proliferation in experimental glomerulonephritis was demonstrated. The aim of the present study is to examine the effect of simvastatin, one of the HMG-CoA reductase inhibitors, on the glomerular cell proliferation and on the expression of CDK2 or p27Kip1 in mesangial cells in experimental glomerulonephritis in vivo. Methods. The effect of simvastatin on a rat mesangial proliferative glomerulonephritis induced by antithymocyte antibody (anti-Thy 1.1 GN) was studied. Administration of simvastatin or vehicle (for control GN) were started from two days before disease induction, and was continued to the day of nephrectomy. Nephrectomy was done at days 0, 2, 4, 7, 12 and 20 after disease induction. Immunohistochemistry for proliferating cells, macrophages, α- smooth muscle actin, type IV collagen and PDGF-B chain was performed, respectively, in addition to conventional periodic-acid Schiff staining. Double immunostaining for CDK2/OX-7 or p27Kip1/OX-7 was also done, respectively. Results. There was no difference in the degree of the initial injuries between simvastatin-treated and control GN rats. The most pronounced feature of simvastatin-treated GN was the suppression of the early glomerular cell proliferation (about 70% of proliferation was suppressed at day 4). At day 4, α-smooth muscle actin expression was also

decreased in simvastatin- treated GN rats. Inhibition of macrophage recruitment into glomeruli by simvastatin was also a prominent feature (about 30% decrease in the number of glomerular macrophages at day 2). Simvastatin significantly suppressed subsequent mesangial matrix expansion and type IV collagen accumulation in glomeruli. Although it might simply reflect the reduction in mesangial cells, glomerular PDGF-B chain expression was reduced. There was no significant difference in plasma lipids levels at day 2 and day 4. In vehicle-treated GN rats, the number of CDK2+/OX-7+ cells (CDK2-expressed mesangial cells) in glomeruli increased significantly from day 4 to day 7. Although simvastatin suppressed mesangial cell proliferation, the increase in the number of glomerular CDK2+/OX-7+ cells was also attenuated by simvastatin treatment. There was no difference in the number of p27Kip1+/OX-7+cells (p27Kip1- expressed mesangial cells) in the glomerulus between vehicle-treated and simvastatin-treated GN rats. Conclusion. Simvastatin suppressed mesangial cell proliferation and subsequent matrix expansion, and macrophage infiltration into glomeruli in anti-Thy 1.1 GN rats. The antiproliferative effect of simvastatin in this model was also associated with the reduction of CDK2 expression in mesangial cells.

L27 ANSWER 8 OF 13 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1998:816569 The Genuine Article (R) Number: 130YD. Simvastatin suppresses glomerular cell proliferation and macrophage infiltration in rats with mesangial proliferative nephritis. Yoshimura A (Reprint); Inui K; Nemoto T; Uda S; Sugenoya Y; Watanabe S; Yokota N; Taira T; Iwasaki S; Ideura T. Showa Univ, Fujigaoka Hosp, Dept Med, Div Nephrol, Aoba Ku, 1-30 Fujigaoka, Yokohama, Kanagawa 227, Japan (Reprint); Showa Univ, Fujigaoka Hosp, Dept Med, Div Nephrol, Aoba Ku, Yokohama, Kanagawa 227, Japan. JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY (NOV 1998) Vol. 9, No. 11, pp. 2027-2039. ISSN: 1046-6673. Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621 USA. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Inhibition of 3-hydro-3-methylglutaryl coenzyme A reductase inhibits the production of mevalonate and has been shown to suppress proliferation in many cell types. Therefore, 3-hydro-3-methylglutaryl coenzyme A reductase inhibitors may have a beneficial effect in glomerular disease, because glomerular cell proliferation is a central feature in the active glomerular injury. This study examines the effect of simvastatin on glomerular pathology in a rat mesangial proliferative glomerulonephritis (GN) induced by anti-thymocyte antibody (anti-Thy1.1 GN). There was no difference in the degree of the antibody and complement-mediated initial injuries between simvastatin-treated and control GN rats. The most pronounced feature of simvastatin-treated GN was the suppression of the early glomerular cell proliferation. The proliferative activity was maximal at day 4 after disease induction (26.5 +/- 7.0 of proliferating cell nuclear antigen-positive cells/glomerulus); however, approximately 70% of proliferation was suppressed by simvastatin treatment. At day 4 after disease induction, simvastatin administration also decreased alpha-smooth muscle actin expression in the glomerulus, which is a marker for mesangial cell activation. Inhibition of monocyte/macrophage recruitment into glomeruli by simvastatin was also a prominent feature. There was a 30% decrease in the number of glomerular ED-1(+) cells by simvastatin treatment at day 2 after disease induction. Furthermore, simvastatin remarkably suppressed subsequent mesangial matrix expansion and type IV collagen accumulation in glomeruli. We also found that the platelet-derived growth factor expression was reduced in simvastatin-treated nephritic rats, which might simply reflect the reduction in mesangial cell proliferation and mesangial cellularity. There was no significant difference in plasma cholesterol or triglyceride levels between simvastatin- and vehicle-treated nephritic rats at day 2 and day 4, which corresponded to the times when simvastatin treatment resulted in a reduction in

mesangial cell proliferation. In conclusion, this is the first report to find that mesangial cell proliferation and matrix expansion have been blocked by simvastatin in vivo. The protective effect of simvastatin in the matrix expansion in anti-Thy1.1 GN was partly by inhibition of mesangial cell proliferation and monocyte/ macrophage recruitment into glomeruli, which were independent of a change in circulating lipids.

L27 ANSWER 9 OF 13 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1998:311538 The Genuine Article (R) Number: ZH989. C-type natriuretic peptide inhibits mesangial cell proliferation and matrix accumulation in vivo. Canaan-Kuhl S; Ostendorf T; Zander K; Koch K M; Floege J (Reprint). Med Hsch Hannover, Div Nephrol 6840, D-30623 Hannover, Germany (Reprint). KIDNEY INTERNATIONAL (MAY 1998) Vol. 53, No. 5, pp. 1143-1151. ISSN: 0085-2538. Publisher: BLACKWELL SCIENCE INC, 350 MAIN ST, MALDEN, MA 02148 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Local C-type natriuretic peptide (CNP) production and CNP receptor expression have been demonstrated in glomeruli. However, the glomerular (patho-)physiological functions of CNP are largely unknown. We therefore investigated the effects of CNP on mesangial cell proliferation and matrix accumulation in the rat mesangioproliferative anti-Thy 1.1 model. Over seven days rats received a continuous infusion (1 mu g/kg/min) of either CNP (N = 6), an irrelevant control peptide (N = 3) or buffer alone (N = 6). Kidney biopsies were performed on days 2, 4 and 8. Few significant differences between the groups were noted on days 2 and 4. Compared to buffer treated rats on day 8, those receiving CNP showed a 35% reduction of glomerular mitoses, a 62% reduction of glomerular uptake of the thymidine analogue BrdU and a significant reduction in glomerular expression of PDGF B-chain. Double immunoperoxidase staining also revealed blunting of proliferating, activated mesangial cells (51% reduction of alpha-smooth muscle actin-/BrdU-positive cells) and macrophage influx, Moreover, there was a marked reduction of mesangial collagen IV and fibronectin accumulation at the protein and mRNA level. Rats receiving the control peptide were indistinguishable from buffer treated rats. Systemic blood pressure was reduced by 10 to 20% in both CNP and control peptide treated rats on day 8, excluding that the findings were due to hemodynamic effects of CNP. Our findings demonstrate that CNP is involved In the regulation of mesangial cell proliferation and matrix production In vivo. The data suggest the existence of a glomerular natriuretic peptide system that may regulate tissue homeostasis and contribute to resolution of mesangioproliferative diseases.

L27 ANSWER 10 OF 13 MEDLINE on STN DUPLICATE 5

1998168334. PubMed ID: 9507491. Pathogenesis of glomerular damage in glomerulonephritis. Couser W G. (Department of Medicine, University of Washington, Seattle 98195, USA.) Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association, (1998) Vol. 13 Suppl 1, pp. 10-5. Ref: 66. Journal code: 8706402. ISSN: 0931-0509. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Although glomerular disease remains the most common cause of end-stage renal disease worldwide, major advances have been made recently in understanding the cellular and molecular mechanisms which mediate these disorders. Nephrotic syndrome in non-inflammatory lesions such as minimal change/focal sclerosis and membranous nephropathy results from disorders of the GEC which can be simulated in animal models by antibodies to various GEC membrane epitopes. Clarification of how these antibodies effect the GEC to induce a loss of glomerular barrier function should substantially improve understanding of the pathogenesis of minimal change/focal sclerosis. In MN, proteinuria is mediated primarily by C5b-9 through similar mechanisms that also involve the GEC as a target and GEC production of oxidants, proteases and TGF beta in response to

sublytic C5b-9 attack. C5b-9 also mediates mesangial proliferative glomerulonephritis induced by anti-measangial cell antibodies and thrombotic microangiopathy induced by antibodies to the glomerular endothelial cell. In all of these lesions induced by antibodies to glomerular cells, cell-bound complement regulatory proteins are important in modulating the injury observed. Upregulation of complement regulatory proteins may prove an effective therapeutic manoeuver in the future. Inflammatory glomerular lesions are induced by circulating inflammatory cells or proliferating resident glomerular cells. Understanding of how these cells induce tissue injury has also evolved considerably over the past decade. Neutrophil-induced disease involves leukocyte adhesion molecules in regulating neutrophil localization; proteases, oxidants and MPO in mediating injury and platelets in augmenting these processes. The activated mesangial cell following immune injury exhibits altered phenotype and proliferation with release of oxidants and proteases. Mesangial cell proliferation may be initiated by bFGF and is maintained by an autocrine mechanism involving PDGF. TGF beta is important in the subsequent development of sclerosis. Finally, recent studies establish the nephritogenic potential of cell-mediated mechanisms alone without humoral immunity, and these mechanisms may be important in glomerulonephritis which occurs in the absence of antibody deposits. As understanding of these areas evolves, numerous new therapeutic strategies can now be devised including agents which selectively block or inhibit complement effects, leukocyte adhesion molecules, oxidants, proteases, growth factors and other cytokines and activated T cells. Appreciation of the role of several natural inhibitors of these mechanisms may also allow therapeutic manipulations that upregulate regulatory proteins with a consequent therapeutic benefit. Thus, these changes in basic understanding of the mechanisms of glomerular disease are likely to translate into new and more specific and effective forms of therapy in the next decade.

L27 ANSWER 11 OF 13 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1994:623747 The Genuine Article (R) Number: PH786. TRAPIDIL INHIBITS HUMAN MESANGIAL CELL-PROLIFERATION - EFFECT ON PDGF BETA-RECEPTOR BINDING AND EXPRESSION. GESUALDO L (Reprint); DIPAOLO S; RANIERI E; SCHENA F P; BRUNACCINI A. UNIV BARI, DIV NEPHROL, I-70124 BARI, ITALY; UNIV BARI, CHAIR NEPHROL, I-70124 BARI, ITALY. KIDNEY INTERNATIONAL (OCT 1994) Vol. 46, No. 4, pp. 1002-1009. ISSN: 0085-2538. Publisher: BLACKWELL SCIENCE INC, 238 MAIN ST, CAMBRIDGE, MA 02142. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Mesangial cell (MC) proliferation, a histopathologic feature common to many human glomerular diseases, is regulated by several growth factors through their binding to specific cell surface receptors. Platelet-derived growth factor (PDGF) is a peptide exerting a potent mitogenic activity on MC. Recently, an increased expression of both PDGF protein and its receptor has been localized in the mesangial areas of several experimental as well as human proliferative glomerulonephritides (GN). Thus, it may be postulated that the inhibition of PDGF action could prevent MC proliferation during mesangial proliferative GN. Trapidil, an antiplatelet drug, has been shown to inhibit the growth of several cell types both in vitro and in vivo. The present study was aimed at evaluating the effect of Trapidil on human MC in vitro. The addition of 100 to 400 mu g/ml Trapidil significantly reduced cell proliferation induced by different growth factors (FCS, PDGF-BB, bFGF, EGF), the highest inhibitory effect being on PDGF-BB stimulated MC growth. The effect of the drug was dose-dependent and seemingly specific: aspirin was devoid of any anti-proliferative action, while dypiridamole proved to be toxic. Receptor binding experiments showed that Trapidil competitively inhibited I-125-PDGF-BB binding to its cell surface receptors, without inducing receptor internalization, at least after short-term (2 hr)

incubation. In contrast, long-term (48 hr) exposure to 400 mu g/ml Trapidil caused a sharp increase of PDGF-BB binding. Similar effects on cell proliferation and I-125-PDGF-BB binding were observed when NIH-3T3 fibroblasts were exposed to the test substance. Finally, we evaluated the influence of Trapidil on PDGF beta-receptor gene expression and found that 24 hour exposure to 400 mu g/ml Trapidil moderately decreased steady-state mRNA levels, whereas 48 hours of incubation increased PDGF beta-receptor transcript levels by approximately threefold. We conclude that Trapidil is able to strongly inhibit human MC proliferation, a process in which inhibition of PDGF-BB binding to its receptors and modulation of PDGF beta-receptor gene expression might be involved.

L27 ANSWER 12 OF 13 MEDLINE on STN DUPLICATE 6
94138205. PubMed ID: 8305838. Mediation of immune glomerular injury. Couser W G. (Department of Medicine, University of Washington, Seattle.) The Clinical investigator, (1993 Oct) Vol. 71, No. 10, pp. 808-11. Ref: 13. Journal code: 9207154. ISSN: 0941-0198. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Although glomerular disease remains the most common cause of end-stage renal disease worldwide, major advances have been made recently in understanding the cellular and molecular mechanisms which mediate these disorders. Nephrotic syndrome in non inflammatory lesions such as minimal-change/focal sclerosis and MN results from disorders of the GEC which can be simulated in animal models by antibodies to various GEC membrane epitopes. Clarification of how these antibodies effect the GEC to induce a loss of glomerular barrier function should substantially improve understanding of the pathogenesis of minimal change/focal sclerosis. In MN, proteinuria is mediated primarily by C5b-9 through similar mechanisms that also involve the GEC as a target. Inflammatory glomerular lesions are induced by circulating inflammatory cells or proliferating resident glomerular cells. Understanding of how these cells induce tissue injury has also evolved considerably over the past decade. Neutrophil-induced disease involves leukocyte adhesion molecules in regulating neutrophil localization; proteases, oxidants, and myeloperoxidase in mediating injury and platelets in augmenting these processes. The activated mesangial cell exhibits altered phenotype and proliferation with release of oxidants and proteases. Mesangial cell proliferation may be initiated by basic fibroblast growth factor and is maintained by an autocrine mechanism involving PDGF. TGF-beta is important in the subsequent development of sclerosis. As understanding of these areas evolves, numerous new therapeutic strategies can now be devised, including agents which block or inhibit complement effects, oxidants, proteases, growth factors, and other cytokines. Appreciation of the role of several natural inhibitors of these mechanisms may also allow therapeutic manipulations that upregulate regulatory proteins, with a consequent therapeutic benefit.(ABSTRACT TRUNCATED AT 250 WORDS)

L27 ANSWER 13 OF 13 EMBASE on STN DUPLICATE 7
93162844 EMBASE Document No.: 1993162844. Heparin suppresses mesangial cell proliferation and matrix expansion in experimental mesangioproliferative glomerulonephritis. Floege J.; Eng E.; Young B.A.; Couser W.G.; Johnson R.J.. Division of Nephrology, University of Washington,Seattle, WA 98195, United States. Kidney International Vol. 43, No. 2, pp. 369-380 1993.
ISSN: 0085-2538. CODEN: KDYIA5
Pub. Country: United States. Language: English. Summary Language: English.
Entered STN: 930704. Last Updated on STN: 930704

AB Proliferation and extracellular matrix (ECM) overproduction by glomerular mesangial cells characterizes many types of glomerulonephritis and often precedes the development of glomerulosclerosis. Heparin is a potent inhibitor of mesangial cell growth in vitro. We examined whether standard heparin can inhibit mesangial cell

proliferation in vivo in the mesangioproliferative anti-Thy 1.1 nephritis. Untreated control rats were compared to rats infused with heparin either early (day -2 to 1) or late (day 2 to 5) after induction of anti-Thy 1.1 nephritis. The results show that heparin treatment significantly reduced mesangial cell proliferation regardless of when it was initiated. Heparin (either early or late treatment) also reduced mesangial basic fibroblast growth factor (bFGF) expression and platelet-derived growth factor (PDGF) receptor up-regulation as reflected by immunostaining, whereas PDGF B-chain expression was reduced only by late heparin treatment. Furthermore, heparin treatment markedly inhibited the mesangial matrix expansion for a variety of ECM proteins, including laminin, type I and IV collagen, fibronectin and entactin. Heparin did not affect the initial mesangiolysis, glomerular macrophage influx, deposition of anti-Thy 1.1 IgG or fibrinogen, or the glomerular platelet influx. These results suggest that heparin, via its antiproliferative rather than anticoagulant effect, can inhibit mesangial cell proliferation, overexpression of polypeptide growth factors, and ECM protein overproduction in vivo. The beneficial effect of heparin can be demonstrated even if treatment is initiated after the development of nephritis. By virtue of these properties, heparin may be an effective agent in the treatment of human mesangioproliferative disease and in the prevention of glomerulosclerosis.

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Brief Definitive Report

Inhibition of Mesangial Cell Proliferation and Matrix Expansion in Glomerulonephritis in the Rat by Antibody to Platelet-derived Growth Factor

By Richard J. Johnson,* Elaine W. Raines,‡ Jürgen Floege,* Ashio Yoshimura,* Pam Pritzl,* Charles Alpers,‡ and Russell Ross‡

*From the *Division of Nephrology, Departments of Medicine and ‡Pathology, University of Washington Medical Center, Seattle, Washington 98195*

Summary

Platelet-derived growth factor (PDGF), a potent mitogen for mesenchymal cells in culture, is expressed in vivo in a variety of inflammatory conditions associated with cell proliferation, including atherosclerosis, wound repair, pulmonary fibrosis, and glomerulonephritis. However, it is not known if PDGF mediates the fibroproliferative responses that characterize these inflammatory disorders. We administered neutralizing anti-PDGF IgG or control IgG to rats with mesangial proliferative nephritis. Inhibition of PDGF resulted in a significant reduction in mesangial cell proliferation, and largely prevented the increased deposition of extracellular matrix associated with the disease. This suggests that PDGF may have a central role in proliferative glomerular disease.

Mesangial cell proliferation and matrix expansion characterize many types of glomerulonephritis (GN). The observations that platelet-derived growth factor (PDGF) is a potent mitogen for mesangial cells in culture (1) and is expressed in both experimental and human GN in which mesangial cell proliferation occurs (2–5) suggest that this factor may have an important role in mediating these changes.

One model in which PDGF has been studied is the mesangial proliferative GN in rats induced by antibody to the Thy-1 antigen, which is expressed by mesangial cells (2). The model is characterized by an acute complement-dependent loss of mesangial cells with disruption of the mesangial matrix ("mesangiolysis") that is maximal at 24 h (2). The mesangial cell population, which is almost completely eliminated, undergoes a rebound proliferation that is accompanied by a marked upregulation of PDGF A and B chain mRNA in total glomerular RNA at 3 and 5 d after disease induction (2). When rats were depleted of either complement or platelets and were injected with anti-Thy-1 antibody, both the glomerular cell proliferation and increased glomerular PDGF expression were significantly inhibited (2, 3). However, despite demonstrating a strong association between glomerular PDGF expression and glomerular cell proliferation in mesangial proliferative GN, these studies do not determine whether PDGF plays a direct role in the pathogenesis of glomerular injury. We now report the effect of blocking PDGF in vivo in this model of nephritis utilizing a neutralizing polyclonal antibody to PDGF.

Materials and Methods

Experimental Protocol. Anti-Thy-1 GN was induced with goat anti-Thy-1 plasma in 150–200-g male Wistar rats (Simonsen, Gilroy, CA) as previously described (2). 8 h before the injection of anti-Thy-1 antibody, rats were injected with goat anti-PDGF IgG (60 mg/100 g body weight, i.p.) (n = 6) or equivalent quantities of nonimmune (control) goat IgG (n = 6) with repeated doses daily for 4 d. After disease induction, rats underwent renal biopsies at 2 and 4 d. Blood samples were collected for serum C3 levels (at 0, 2, and 4 d), leukocyte and platelet counts (day 4), and plasma anti-PDGF IgG levels (day 4, measured by ELISA [6]).

Anti-PDGF Antibody. The anti-PDGF IgG was raised in a goat immunized with PDGF purified from outdated human platelets and specifically neutralizes the mitogenic activity of rat PDGF and all dimeric forms of human PDGF (6).

Histology. The following antibodies were used for immunoperoxidase staining of methyl Carnoy's fixed, paraffin-embedded tissue: 19A2 (Coulter Immunology, Hialeah, FL), a mAb to the proliferating cell nuclear antigen (PCNA), which is a cell proliferation marker; ED-1 (Bioproducts for Science, Indianapolis, IN), a mAb to rat monocytes and macrophages; rabbit anti–rat collagen I and rabbit anti–rat laminin (Chemicon, Temecula, CA); rabbit anti–mouse collagen IV (Collaborative Research Inc., Bedford, MA); rabbit anti–mouse entactin (gift of A. Chung, Pittsburgh, PA); and rabbit anti–mouse heparan sulfate proteoglycan (gift of J. Couchman, Birmingham, AL) (2, 7). Immunofluorescence of snap-frozen tissue was also performed using FITC-conjugated rabbit anti–goat IgG and FITC-conjugated goat anti–rat C3 antibodies (Organon Teknika Corp., West Chester, PA) (8). Histological changes were quantitated as previously described (2, 7). PDGF B

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chain mRNA was detected in formalin-fixed tissue using a digoxigenin-labeled antisense cRNA probe and quantitated as previously described (3).

Statistics. Values are expressed as mean ± SD. The one-tailed student's *t* test was used to test the hypothesis that anti-PDGF treatment would reduce cell proliferation, total glomerular cellularity, or matrix expansion relative to controls.

Results and Discussion

In this study, neutralizing anti-PDGF IgG or control IgG was administered to rats with mesangial proliferative GN. Two time points were selected for study, representing the day of initial proliferation (day 2) and the day of peak proliferation (day 4). Later time points were not studied due to the concern that the rats would develop autoantibodies to the administered goat IgG.

Anti-PDGF IgG treatment was well tolerated, and serum C3 levels (measured at 0, 2, and 4 d) and platelet and leukocyte counts (at 4 d) were normal and not different from controls. Whereas anti-PDGF IgG levels were undetectable in control rats, plasma anti-PDGF IgG levels in treated rats were 4.6 ± 0.7 mg/ml at day 4, concentrations that are 20–30 times that required to inhibit the mitogenic activity of PDGF on rat smooth muscle cells in vitro (6).

Previous studies have demonstrated that the initial injury (i.e., mesangiolysis) in this model is dependent on delivery and binding of anti-Thy-1 antibody to the mesangial cell followed by complement activation (9). Anti-PDGF IgG treatment did not prevent this initial injury, as both control and anti-PDGF IgG–treated rats had equivalent mesangiolysis (2.6 ± 0.5 vs. 2.9 ± 0.2, respectively, scale of 0–4+; p = NS) with an equal reduction in total glomerular cellularity at day 2 (Table 1). Anti-PDGF IgG treatment also did not inhibit the glomerular macrophage infiltration at either day 2 (9.1 ± 2 vs. 9.2 ± 1) or day 4 (8.0 ± 2 vs. 8.8 ± 1 ED-1$^+$ cells/glomerular cross-section in control vs. anti-PDGF IgG–treated rats, respectively).

Table 1. *Effect of Anti-PDGF IgG Treatment on Total Glomerular Cellularity and Proliferating (PCNA$^+$) Cells in Mesangial Proliferative GN.*

	Total cells	Proliferating (PCNA$^+$) cells
Normal	77 ± 1.8	0.9 ± 0.2
Mesangial proliferative GN, day 2		
Control	53 ± 5	10.4 ± 1
Anti-PDGF	53 ± 6	10.0 ± 2
Mesangial proliferative GN, day 4		
Control	89 ± 13	13.4 ± 4
Anti-PDGF	77 ± 10*	5.7 ± 1‡

Values are expressed as the mean number ± SD of cells per glomerular cross-section. For comparison, values for normal Wistar rats (n = 6) are shown (2).
* p <0.05 relative to control rats.
‡ p <0.005 relative to control rats.

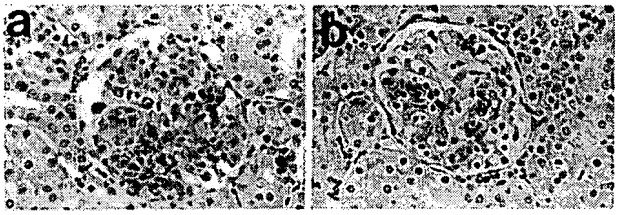

Figure 1. Compared with control rats with mesangial proliferative GN (*a*), anti-PDGF IgG–treated rats with GN (*b*) had significantly less glomerular cellularity at day 4 (periodic acid/Schiff reagent with hematoxylin counterstain, ×240).

Effect on Glomerular Cell Proliferation. The initial glomerular cell proliferation (i.e., PCNA$^+$ cells) was not affected by anti-PDGF IgG treatment (Table 1). In contrast, at day 4, during the peak phase of cell proliferation, a 57% reduction in cell proliferation was observed ($p < 0.005$), and was associated with a significant reduction in total glomerular cellularity as compared with controls (Table 1 and Fig. 1). Most of the proliferating cells in this model have been shown to be mesangial cells (2, 8), and this was supported in the current study in which >85% of the PCNA$^+$ cells in both groups excluded the monocyte-macrophage marker, ED-1, by double immunolabeling.

One concern was that anti-PDGF IgG treatment might result in significant PDGF/anti-PDGF immune complexes in glomeruli that would prolong the mesangiolysis and delay the proliferative response. However, both control and anti-PDGF IgG–treated rats had equivalent mesangial staining of IgG at 2 and 4 d (2+, scale of 0–4+) with negative staining of C3 by immunofluorescence. Similarly, incubation of both resting and proliferating mesangial cells in culture with anti-PDGF IgG (5 mg/ml) resulted in no significant ^{51}Cr release compared with control IgG in the presence of complement (data not shown). Finally, injection of equivalent doses of anti-PDGF IgG into normal rats (n = 2) as used in the experimental study resulted in no detectable mesangial injury in biopsies obtained at days 2 and 4.

The observation that mesangial cell proliferation was reduced by anti-PDGF IgG treatment at day 4 but not at day 2 has several potential interpretations, including: (*a*) the possibility that numerous growth factors, including PDGF, are involved in the initial cell proliferation such that mesangial cells are maximally stimulated even when PDGF is inhibited; (*b*) the possibility that inadequate anti-PDGF IgG was present locally to block PDGF before its binding to the mesangial cell at day 2; and (*c*) the possibility that responsiveness to PDGF requires upregulation of the PDGF receptor. PDGF receptor β subunit expression is known to be upregulated in this disease (2), and in this study, PDGF receptor β subunit immunostaining was markedly increased in glomeruli at day 4 relative to day 2 (data not shown).

Other possible growth regulatory molecules that could mediate the initial mesangial cell proliferation include factors

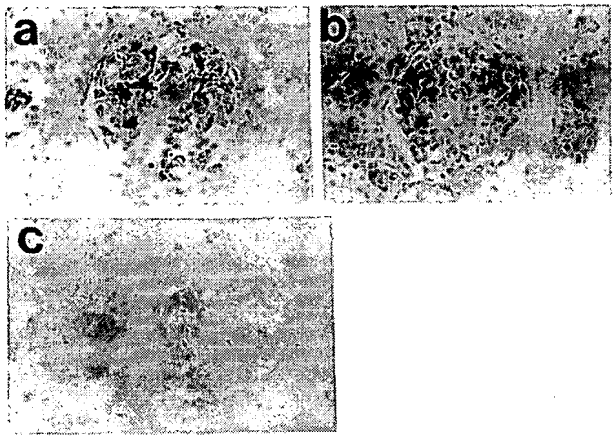

Figure 2. Whereas the expression of PDGF B chain mRNA was often diffuse in glomeruli of control rats with mesangial proliferative GN (*a*), in anti-PDGF IgG–treated rats with GN, expression was often segmental (*b*). Hybridization with a sense probe for PDGF B chain mRNA was always negative (*c*) (×240).

released by platelets, since thrombocytopenic animals show a decrease in mesangial cell proliferation in this model at day 3 (8) and basic fibroblast growth factor (bFGF), which is released by a variety of damaged cells, and is a mitogen for mesangial cells in culture (10). Recently, we have shown that mesangial cells produce bFGF, and release bFGF during acute mesangiolysis (i.e., the first 24 h) in this model (J. Floege et al., manuscript submitted for publication). This may also be relevant to the PDGF-mediated response, given that bFGF will induce both PDGF expression by mesangial cells in culture (10) and an increase in PDGF receptors on smooth muscle cells (our unpublished observations).

Glomerular Source of PDGF. Although the source of the glomerular PDGF may partially originate from platelets, activated macrophages, or endothelial cells, our previous studies (2, 3) suggest that most of the PDGF is expressed by mesangial cells, where it may function as an autocrine growth factor.

Table 2. *Effect of Anti-PDGF IgG Treatment on Extracellular Matrix Accumulation in Mesangial Proliferative GN*

	Normal	Control	Anti-PDGF
Type IV collagen	0.9 ± 0.8	3.1 ± 0.7	2.1 ± 0.4*
Type I collagen	0.03 ± 0.02	2.5 ± 0.6	1.6 ± 0.4*
Laminin	0.8 ± 0.4	2.6 ± 0.6	1.8 ± 0.3*
Entactin/nidogen	0.2 ± 0.1	2.5 ± 0.7	1.6 ± 0.4*
Heparan sulfate proteoglycan	1.8 ± 0.5	3.1 ± 0.6	2.6 ± 0.6

Semiquantitative immunohistochemical scores for various ECM components (scale of 0–4+ [7]) in the mesangium of rats with mesangial proliferative GN that had been treated with nonimmune IgG (control, $n = 6$) or with anti-PDGF IgG ($n = 6$). For comparison, the values in normal Wistar rats ($n = 6$) are shown.
* $p < 0.01$ vs. control rats.

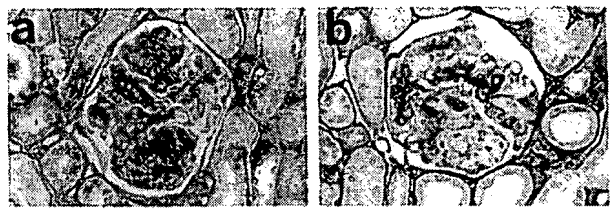

Figure 3. A diffuse increase in laminin was present in the mesangium of control rats with GN (*a*), and was significantly reduced in rats with GN that had received anti-PDGF IgG (*b*) (×240).

We therefore performed in situ hybridization to determine the effect of anti-PDGF IgG treatment on PDGF B chain mRNA expression at day 4. An increase in PDGF B chain mRNA could be detected in mesangial regions in both control and anti-PDGF IgG–treated rats (Fig. 2). However, the amount of PDGF B chain mRNA in the glomeruli was lower in anti-PDGF IgG–treated animals (1.68 ± 0.4 vs. 1.24 ± 0.2 in control vs. anti-PDGF-treated rats, scale of 0–3+, one-tailed Student's *t* test; $p < 0.05$ [3]). The reduction in glomerular expression of PDGF B chain mRNA in the anti-PDGF IgG–treated rats is consistent with an inhibition of mesangial cell proliferation and an interruption of PDGF-mediated induction of its own expression in mesangial cells (10).

Effect of Anti-PDGF IgG Treatment on Extracellular Matrix (ECM) Accumulation. Previous studies have demonstrated that in this model mesangial cell proliferation is accompanied by an expansion of several ECM proteins in the mesangium (7, 11). In this study, control rats with anti-Thy-1 GN also had a diffuse increase in glomerular staining at day 4 for various ECM proteins, including types I and IV collagen, laminin, and entactin (Table 2). In contrast, glomeruli from anti-PDGF IgG–treated rats with GN showed significantly less, and often only segmental, increases in staining (Table 2 and Fig. 3).

The reduction in immunostaining for the various ECM components in anti-PDGF IgG–treated rats may reflect, in part, the reduction in mesangial cell number. Alternatively, anti-PDGF treatment may be affecting TGF-β production within the glomeruli. PDGF induces mesangial cells to express TGF-β (12). TGF-β, in turn, induces mesangial cells to produce a variety of ECM components (13, 14). TGF-β is also increased in glomeruli of rats with anti-Thy-1 GN, and treatment of rats with anti-Thy-1 GN with anti-TGF-β antibody inhibits expansion of the mesangial matrix (11). Thus, it is possible that the beneficial effects of anti-PDGF treatment on ECM expansion in anti-Thy-1 GN may reflect inhibition of PDGF-mediated stimulation of mesangial cell production of TGF-β.

In conclusion, the current study provides the first direct in vivo evidence for a role for PDGF as a growth stimulatory molecule in GN. Treatment of rats with mesangial proliferative GN with a neutralizing anti-PDGF antibody significantly reduced mesangial cell proliferation and matrix expansion at 4 d. The observation that the inhibition was only partial (i.e., 60%) suggests that other growth factors may be involved in this proliferative response, or that there was insufficient antibody available at the cellular level to effect a total response.

The fact that this degree of proliferation and matrix expansion could be reduced by inhibiting a single growth factor suggests that PDGF may play a crucial role in progressive glomerular injury.

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Address correspondence to Richard J. Johnson, Division of Nephrology, RM-11, Department of Medicine, School of Medicine, University of Washington, Seattle, WA 98195.

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Novel Approach to Specific Growth Factor Inhibition *in Vivo*

Antagonism of Platelet-Derived Growth Factor in Glomerulonephritis by Aptamers

Jürgen Floege,* Tammo Ostendorf,* Ulf Janssen,* Michael Burg,* Heinfried H. Radeke,† Chandra Vargeese,‡ Stanley C. Gill,‡ Louis S. Green,‡ and Nebojša Janjić‡

From the Divisions of Nephrology and Clinical Pharmacology,† Medizinische Hochschule, Hannover, Germany, and NeXstar Pharmaceuticals,‡ Boulder, Colorado*

Mesangial cell proliferation and matrix accumulation, driven by platelet-derived growth factor (PDGF), contribute to many progressive renal diseases. In a novel approach to antagonize PDGF, we investigated the effects of a nuclease-resistant high-affinity oligonucleotide aptamer *in vitro* and *in vivo*. In cultured mesangial cells, the aptamer markedly suppressed PDGF-BB but not epidermal- or fibroblast-growth-factor-2-induced proliferation. *In vivo* effects of the aptamer were evaluated in a rat mesangioproliferative glomerulonephritis model. Twice-daily intravenous (i.v.) injections from days 3 to 8 after disease induction of 2.2 mg/kg PDGF-B aptamer, coupled to 40-kd polyethylene glycol (PEG), led to 1) a reduction of glomerular mitoses by 64% on day 6 and by 78% on day 9, 2) a reduction of proliferating mesangial cells by 95% on day 9, 3) markedly reduced glomerular expression of endogenous PDGF B-chain, 4) reduced glomerular monocyte/macrophage influx on day 6 after disease induction, and 5) a marked reduction of glomerular extracellular matrix overproduction (as assessed by analysis of fibronectin and type IV collagen) both on the protein and mRNA level. The administration of equivalent amounts of a PEG-coupled aptamer with a scrambled sequence or PEG alone had no beneficial effect on the natural course of the disease. These data show that specific inhibition of growth factors using custom-designed, high-affinity aptamers is feasible and effective. *(Am J Pathol 1999, 154:169–179)*

Specific inhibition of growth factors and cytokines has become a major goal in experimental and clinical medicine. However, this approach is often hampered by the lack of specific pharmacological antagonists. Available alternative approaches are also limited, as neutralizing antibodies often show a low efficacy *in vivo* and may be immunogenic, and as *in vivo* gene therapy for these purposes is still in its infancy. In the present study we have investigated a novel approach to specifically inhibit growth factors *in vivo*, namely, the use of aptamers produced by the systematic evolution of ligands by exponential enrichment (SELEX) method.[1,2] The SELEX method has recently emerged as a powerful tool for screening large sequence-randomized nucleic acid libraries for unique oligonucleotides (aptamers) that bind to various other molecules with high affinity and specificity. For the purpose of this study, we have targeted platelet-derived growth factor (PDGF), the role of which is particularly well established in cardiovascular and renal disease.[3,4]

A large variety of progressive renal diseases is characterized by glomerular mesangial cell proliferation and matrix accumulation.[5] PDGF B-chain appears to have a central role in driving both of these processes given that 1) mesangial cells produce PDGF *in vitro* and various growth factors induce mesangial cell proliferation via induction of auto- or paracrine PDGF B-chain excretion, 2) PDGF B-chain and its receptor are overexpressed in many glomerular diseases, 3) infusion of PDGF-BB or glomerular transfection with a PDGF B-chain cDNA can induce selective mesangial cell proliferation and matrix accumulation *in vivo*, and 4) PDGF B-chain or β-receptor knock-out mice fail to develop a mesangium (reviewed in Ref. 4).

So far only one study has examined the effect of inhibition of PDGF B-chain in renal disease; Johnson et al,

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J. Floege and T. Ostendorf contributed equally to this study.

Address reprint requests to Dr. Jürgen Floege, Division of Nephrology 6840, Medizinische Hochschule, 30623 Hannover, Germany (E-mail: Floege.Juergen@MH-Hannover.de) or Nebojsa Janjic, Ph.D., NeXstar Pharmaceuticals, Inc., 2860 Wilderness Place, Boulder, CO 80301; E-mail: njanjic@nexstar.com.

using a neutralizing polyclonal antibody to PDGF, were able to reduce mesangial cell proliferation and matrix accumulation in a rat model of mesangioproliferative glomerulonephritis.[6] In this model, injection of an anti-mesangial cell antibody (anti-Thy-1.1) results in complement-dependent lysis of the mesangial cells, followed by an overshooting reparative phase that resembles human mesangioproliferative nephritis.[7] Limitations of the study of Johnson et al[6] included the necessity to administer large amounts of heterologous IgG and a limitation of the study duration to 4 days due to concerns that the heterologous IgG might elicit a humoral immune reaction. In the present study we have therefore used the anti-Thy-1.1 nephritis model to evaluate the feasibility and efficacy of inhibiting PDGF B-chain *in vivo* with high-affinity DNA-based aptamers.

Materials and Methods

Synthesis of High-Affinity DNA-Based Aptamers to the PDGF B-Chain

All aptamers and their sequence-scrambled controls were synthesized by the solid-phase phosphoramidite method on controlled pore glass using an 8800 Milligen DNA synthesizer and deprotected using ammonium hydroxide at 55°C for 16 hours. 2-Fluoropyrimidine nucleoside phosphoramidites were obtained from JBL Scientific (San Luis Obispo, CA). 2′-*O*-Methylpurine phosphoramidites were obtained from PerSeptive Biosystems (Boston, MA). Hexaethylene glycol (18-atom) spacer was obtained from Glen Research (Sterling, VA). All other nucleoside phosphoramidites were from PerSeptive Biosystems. To prolong the *in vivo* half-time of the aptamers in plasma, they were coupled to 40-kd polyethylene glycol (PEG). The covalent coupling of PEG to the aptamer (or to its sequence-scrambled control) was accomplished by treating 40-kd PEG *N*-hydroxysuccinimide ester (Shearwater Polymers, Huntsville, AL) with a primary amine group introduced at the 5′ end of the aptamer using trifluoroacetyl-protected pentylamine phosphoramidite. All PEG-aptamer conjugates were purified by anion exchange followed by reverse-phase high-pressure liquid chromatography (HPLC). The binding affinities of various aptamers for PDGF-AB or -BB (R&D Systems, Minneapolis, MN) were determined by the nitrocellulose filter binding method[8] or by the competition electrophoresis mobility shift assay.[9]

Cloning and Expression of Rat PDGF-BB

Rat PDGF-BB for cross-reactivity binding experiments was derived from *Escherichia coli* transfected with sCRScript Amp SK(+) plasmid containing the rat PDGF-BB sequence. Rat PDGF-BB sequence was derived from rat lung poly A+ RNA (Clontech, San Diego, CA) through reverse transcription polymerase chain reaction (RT-PCR) using primers that amplify sequence encoding the mature form of PDGF-BB. Rat PDGF-BB protein expression and purification was performed at R&D Systems.

Stability of Aptamers in Rat Plasma in Vitro and in Vivo

The stabilities of DNA-based aptamers *in vitro* were examined in rat serum at 37°C. Serum was obtained from a Sprague-Dawley rat and was filtered through a 0.45-μm cellulose acetate filter and buffered with 20 mmol/L sodium phosphate buffer. Test ligands were added to the serum at the final concentration of 500 nmol/L. The final serum concentration was 85% as a result of the addition of buffer and aptamer. From the original 900-μl incubation mixture, 100-μl aliquots were withdrawn at various time points and added to 10 μl of 500 mmol/L EDTA (pH 8.0), mixed and frozen on dry ice, and stored at −20°C until the end of the experiment. The amount of full-length oligonucleotide ligand remaining at each of the time points was quantitated by HPLC analysis. To prepare the samples for HPLC injections, 200 μl of a mixture of 30% formamide, 70% 25 mmol/L Tris buffer (pH 8.0) containing 1% acetonitrile was added to 100 μl of thawed time point samples, mixed for 5 seconds, and spun for 20 minutes at 14,000 rpm in an Eppendorf microcentrifuge. The analysis was performed using an anion exchange chromatography column (NuceoPac, Dionex, PA-100, 4 × 50 mm) applying a LiCl gradient. The amount of full-length oligonucleotide remaining at each time point was determined from the peak areas.

Pharmacokinetics of the Modified PDGF Aptamer Conjugated to 40-kd PEG in Vivo

The pharmacokinetic properties of the modified PDGF aptamer conjugated to 40-kd PEG were determined in Sprague-Dawley rats. Before animal dosing, the aptamer was diluted with sterile PBS from a stock solution (also in sterile PBS), to final concentrations between 1 and 2 mg/ml (based on oligonucleotide molecular weight and the ultraviolet absorption at 260 nm with an extinction coefficient of 0.037 per mg oligo/ml). A single dose of the aptamer was administered to three Sprague-Dawley rats by i.v. bolus injection through the tail vein. Blood samples (approximately 400 μl) were obtained by venipuncture under isofluorane anesthesia and placed in EDTA-containing tubes. The EDTA blood samples were immediately processed by centrifugation to attain plasma and stored frozen at ≤−20°C. Time points for blood sample collection ranged from 2 to 480 minutes

To prepare plasma samples for HPLC analysis, 200 μl of methanol was added to 100 μl of plasma sample, mixed for 30 seconds, and spun in a centrifuge for 10 minutes at 15,000 × *g*. All of the supernatant was transferred to a new tube and dried under vacuum. Samples were resuspended by addition of 100 μl of 50% formamide and 4% perchloric acid, mixed, and spun as described above. Ninety microliters of supernatant was transferred to a 250-μl limited-volume insert vial for HPLC analysis. Samples were analyzed by anion exchange HPLC (DNAPac PA-100, 4 × 50 mm) with LiCl gradient elution in 30% formamide and monitoring of ultraviolet absorption at 270 nm. Aptamer concentrations were de-

termined based on peak area from a standard curve of the PEG-aptamer conjugate. The HPLC-based analysis described here and the double hybridization analysis described previously[9] have produced comparable results for a similar aptamer conjugated to 40-kd PEG (specific binding to vascular endothelial growth factor, Stanley C. Gill, unpublished observations), suggesting that the HPLC analysis indicates the levels of undegraded (full-length) aptamer. Nevertheless, as the PEG moiety has a strong influence on the HPLC retention times of the PEG-aptamer conjugate, we have not at this time ruled out the possibility that some of the partially degraded aptamer may migrate with a similar retention time as the full-length aptamer.

Mesangial Cell Culture Experiments

Human and rat mesangial cells were established in culture, characterized, and maintained as described previously.[10] To examine the antiproliferative effect of the aptamers on the cultured mesangial cells, cells were seeded in 96-well plates (Nunc, Wiesbaden, Germany) and grown to subconfluency. They were then growth-arrested for 48 hours in MCDB 302 medium (Sigma, Deisenhofen, Germany) (human mesangial cells) or RPMI 1640 with 1% bovine serum albumin (rat mesangial cells). After 48 hours, various stimuli together with PDGF B-chain aptamer or sequence-scrambled aptamer were added: medium alone, human recombinant PDGF-AA, -AB, or -BB (kindly provided by J. Hoppe, University of Würzburg, Germany), human recombinant epidermal growth factor (EGF; Calbiochem, Bad Soden, Germany), or recombinant human fibroblast growth factor-2 (kindly provided by Synergen/Amgen, Boulder, CO). DNA synthesis in the rat mesangial cells was determined by [^{3}H]thymidine incorporation as described[11] after 24 hours of stimulation (of which the last 4 hours were in the presence of [^{3}H]thymidine). In the case of human mesangial cells, after 72 hours of incubation, numbers of viable cells were determined using 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT; Sigma) as described.[12]

Experimental Design

All animal experiments were approved by the local review boards. Anti-Thy-1.1 mesangial proliferative glomerulonephritis was induced in 33 male Wistar rats (Charles River, Sulzfeld, Germany) weighing 150 to 160 g by injection of 1 mg/kg monoclonal anti-Thy-1.1 antibody (clone OX-7; European Collection of Animal Cell Cultures, Salisbury, UK). Rats were treated with aptamers or PEG (see below) from days 3 to 8 after disease induction. Treatment consisted of twice-daily i.v. bolus injections of the substances dissolved in 400 μl of PBS, pH 7.4, for a total of 12 injections. The treatment duration was chosen to treat rats from ~1 day after the onset to the peak of mesangial cell proliferation, which in the OX-7-induced anti-Thy-1.1 nephritis occurs between days 6 and 9 after disease induction. Four groups of rats were studied: 1) 9 rats that received a total of 4 mg (0.33 mg/injection) of the PDGF-B aptamer (coupled to 15.7 mg 40 kd PEG), 2) 10 rats that received an equivalent amount of PEG-coupled, scrambled aptamer, 3) 8 rats that received an equivalent amount (15.7 mg) of 40-kd PEG alone, and 4) 6 rats that received 400-μl bolus injections of PBS alone. Renal biopsies for histological evaluation were obtained on day 6 by intravital biopsy and postmortem on day 9 after disease induction. For intravital biopsies the left kidney was exposed by a flank incision under general anesthesia. A 3- to 4-mm slice was then cut off the lower pole, and bleeding was stopped immediately by gently applying a collagen sponge, followed by wound closure. As judged from serum creatinines, this biopsy technique does not disturb renal function (unpublished observations). Twenty-four-hour urine collections were performed from days 5 to 6 and 8 to 9 after disease induction. The thymidine analogue 5-bromo-2'-deoxyuridine (BrdU; Sigma; 100 mg/kg body weight) was injected intraperitoneally at 4 hours before sacrifice on day 9.

Normal ranges of proteinuria and renal histological parameters (see below) were established in 10 nonmanipulated Wistar rats of similar age.

Renal Morphology

Tissue for light microscopy and immunoperoxidase staining was fixed in methyl Carnoy's solution[13] and embedded in paraffin. Four-micron sections were stained with the periodic acid Schiff (PAS) reagent and counterstained with hematoxylin. In the PAS-stained sections the number of mitoses within 100 glomerular tufts was determined.

Immunoperoxidase Staining

Four-micron sections of methyl Carnoy's-fixed biopsy tissue were processed by an indirect immunoperoxidase technique as described.[13] Primary antibodies were identical to those described previously[14,15] and included a murine monoclonal antibody (clone 1A4) to α-smooth muscle actin; a murine monoclonal antibody (clone PGF-007) to PDGF B-chain; a murine monoclonal IgG antibody (clone ED1) to a cytoplasmic antigen present in monocytes, macrophages, and dendritic cells; affinity-purified polyclonal goat anti-human/bovine type IV collagen IgG preabsorbed with rat erythrocytes; an affinity-purified IgG fraction of a polyclonal rabbit anti-rat fibronectin antibody; plus appropriate negative controls as described previously.[14,15] Evaluation of all slides was performed by an observer who was unaware of the origin of the slides.

To obtain mean numbers of infiltrating leukocytes in glomeruli, more than 50 consecutive cross sections of glomeruli were evaluated and mean values per kidney were calculated. For the evaluation of the immunoperoxidase stains for α-smooth muscle actin and PDGF B-chain, each glomerular area was graded semiquantitatively, and the mean score per biopsy was calculated. Each score reflects mainly changes in the extent rather than intensity of staining and depends on the percentage

of the glomerular tuft area showing focally enhanced positive staining: I, 0% to 25%; II, 25% to 50%; III, 50% to 75%; IV, >75%. We have recently described that data obtained using this scoring system are highly correlated with those obtained by computerized morphometry.[16,17]

Immunohistochemical Double Staining

Double immunostaining for the identification of the type of proliferating cells was performed as reported previously[16,17] by first staining the sections for proliferating cells with a murine monoclonal antibody (clone BU-1) against bromodeoxyuridine-containing nuclease in Tris-buffered saline (Amersham, Braunschweig, Germany) using an indirect immunoperoxidase procedure. Sections were then incubated with the IgG_1 monoclonal antibodies 1A4 against α-smooth muscle actin and ED1 against monocytes/macrophages. Cells were identified as proliferating mesangial cells or monocytes/macrophages if they showed positive nuclear staining for BrdU and if the nucleus was completely surrounded by cytoplasm positive for α-smooth muscle actin or ED1 antigen. Negative controls included omission of either of the primary antibodies, in which case no double staining was noted.

In Situ *Hybridization for Type IV Collagen mRNA*

In situ hybridization was performed on 4-μm sections of biopsy tissue fixed in buffered 10% formalin using a digoxigenin-labeled antisense RNA probe for type IV collagen[18] as described.[14] Detection of the RNA probe was performed with an alkaline-phosphatase-coupled anti-digoxigenin antibody (Genius nonradioactive nucleic acid detection kit, Boehringer-Mannheim, Mannheim, Germany) with subsequent color development. Controls consisted of hybridization with a sense probe to matched serial sections by hybridization of the antisense probe to tissue sections that had been incubated with RNAse A before hybridization or by deletion of the probe, antibody, or color solution.[15] Glomerular mRNA expression was semiquantitatively assessed using the scoring system described above.

Miscellaneous Measurements

Urinary protein was measured using the Bio-Rad protein assay (Bio-Rad Laboratories, München, Germany) and bovine serum albumin (Sigma) as a standard.

Statistical Analysis

All values are expressed as means ± SD. Statistical significance (defined as $P < 0.05$) was evaluated using Student *t*-tests or analysis of variance and Bonferroni *t*-tests.

Results

Post-SELEX Modifications in PDGF DNA Aptamers Result in Improved Nuclease Resistance

High-affinity DNA aptamers to the PDGF B-chain were identified previously by the SELEX process.[8] The consensus secondary structure motif of these aptamers is a three-way helix junction with a conserved single-stranded loop at the point of strand exchange (Figure 1A). To improve nuclease resistance of one of the minimal aptamers (a truncated version of aptamer 36t in Ref. 8), we have synthesized and tested a series of 2′-*O*-methyl- or 2′-fluoro-substituted aptamers to identify positions that tolerate such substitutions without a loss of binding affinity. In addition to identifying a pattern of allowed 2′-*O*-methyl- and 2′-fluoro substitutions, we found that trinucleotide loops on helices II and III in the aptamer could be replaced with hexaethylene glycol (18-atom) non-nucleotide spacers without compromising high-affinity binding to PDGF-AB or -BB (Figure 1A). This finding is in agreement with the notion that the helix junction domain of the aptamer represents the core of the structural motif required for high-affinity binding.[8] In practical terms, the replacement of six nucleotides with two spacers is advantageous in that it reduces by four the number of coupling steps required for the synthesis of the aptamer. Another support for the importance of helix junction domain in binding comes from the control aptamer, in which eight nucleotides in the helix junction region were interchanged without formally changing the consensus secondary structure (Figure 1A). The binding affinity of this scrambled aptamer for PDGF-BB ($K_d \approx 1$ μmol/L) is 10,000-fold lower compared with the binding affinity of the aptamer used in the experiments described below ($K_d \approx 0.1$ nmol/L).

We next compared the stabilities of the modified aptamer and its precursor DNA aptamer in rat serum *in vitro*. The half-time of the modified aptamer in serum was considerably longer (~8 hours) compared with that of its all-DNA precursor (~0.6 hours; Figure 1B). The observed increase in nuclease resistance is in agreement with previous studies with 2′-substituted nucleic acids.[19,20] In addition to the modifications mentioned above, for all experiments reported here, the modified DNA aptamer was conjugated to 40-kd PEG. Importantly, the addition of the PEG moiety to the 5′ end of the aptamer has no effect on the binding affinity of the aptamer for PDGF-BB ($K_d \approx 0.1$ nmol/L).

Pharmacokinetics of the Modified PDGF Aptamer Conjugated to 40-kd PEG in Vivo

The concentration of the modified PDGF aptamer conjugated to 40-kd PEG in rat plasma after i.v. injection (1 mg/kg) is shown in Figure 1C. The clearance of the aptamer-PEG conjugate from plasma is biphasic with approximately 47% of the compound being cleared with a half-life of 32.4 ± 13 minutes and 53% of the compound

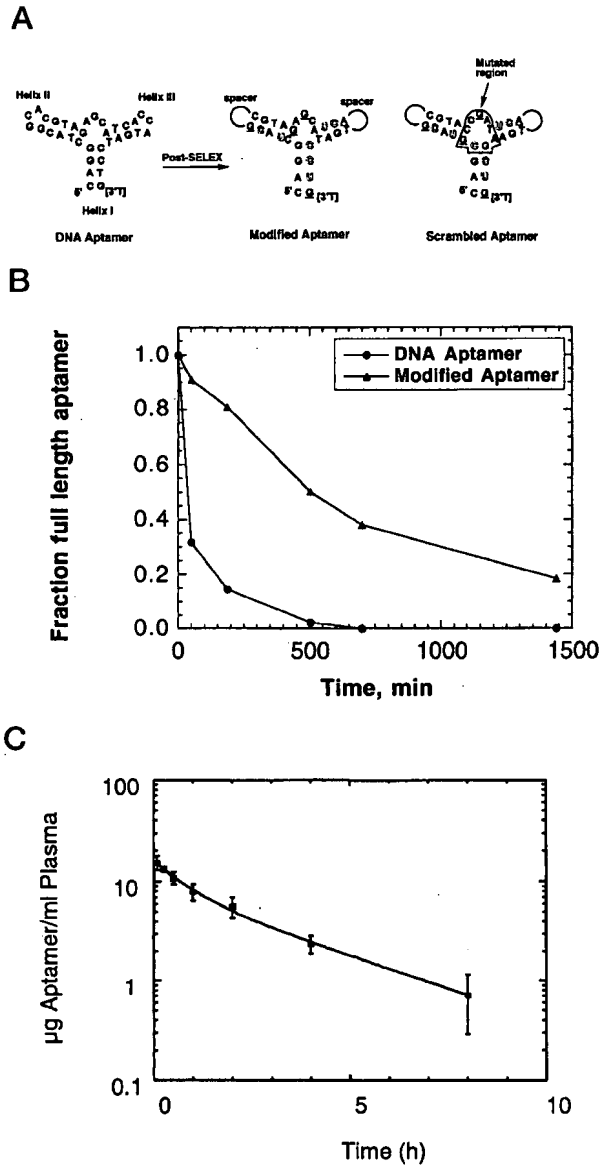

Figure 1. A: Summary of post-SELEX modifications in the PDGF B-chain aptamers. Outlined and underlined letters denote 2′-fluoro and 2′-*O*-methyl nucleotides, respectively; spacer indicates the hexaethylene glycol linker, and [3′T] indicates an inverted (3′-3′) thymidine nucleotide used as a cap to reduce 3′ to 5′ exonuclease-mediated digestion. The mutated region of the scrambled region is boxed to accent the overall similarity to the aptamer. B: Stability of the all-DNA aptamer and the modified PDGF B-chain aptamers in rat serum at 37°C *in vitro*. C: Plasma concentration of modified PDGF-B aptamer conjugated to 40-kd PEG in Sprague-Dawley rats (mean ± SD; n = 3). Compartmental pharmacokinetic analysis (curve fit) was carried out using WinNonlin, version 1.5 (Scientific Consulting Apex, NC).

being cleared with a half-life of 134.5 ± 13 minutes. The concentration of the aptamer in rat plasma after the i.v. injection is 16 µg/ml (1.6 µmol/L) at t = 0 and 0.21 µg/ml (21 nmol/L) at t = 12 hours (extrapolated). Thus, to a first approximation and assuming a linear increase in plasma concentration of the aptamer at the higher injected dose in the experiments described below (2.1 to 2.2 mg/kg i.v. every 12 hours), the aptamer concentration during the treatment period was not lower than 40 nmol/L (ie, 0.4 µg/ml).

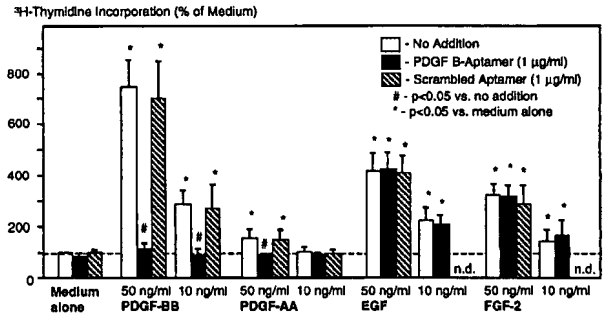

Figure 2. Effects of PDGF-B aptamer on mitogen-stimulated DNA synthesis of rat mesangial cells in culture. Data are 4-hour [^{3}H]thymidine incorporation rates and are expressed as percentage of baseline incorporation (medium alone with no addition, 1373 ± 275 cpm). Data are means ± SD of five independent experiments.

Cross-Reactivity of Aptamers for Rat PDGF-BB

The sequence of PDGF is highly conserved among species, and human and rat PDGF B-chain sequences are 89% identical.[21,22] Nevertheless, in view of the high specificity of aptamers,[23] the correct interpretation of the *in vivo* experiments requires understanding of the binding properties of the aptamers to rat PDGF B-chain. We have therefore cloned and expressed the mature form of rat PDGF-BB in *E. coli*. Based on nitrocellulose filter binding experiments, the PDGF aptamers bound to rat and human recombinant PDGF-BB with the same affinity ($K_d \approx$ 0.1 nmol/L).

PDGF B-Chain DNA-Aptamer Specifically Inhibits PDGF-BB-Induced Rat and Human Mesangial Cell Proliferation in Vitro

In growth-arrested rat mesangial cells, the effects of the PDGF-B aptamer or the scrambled aptamer on mitogen-induced proliferation were tested. Stimulated growth rates of the cells were not affected by the addition of scrambled aptamer (Figure 2). At a concentration of 1 µg/ml the PDGF-B aptamer completely inhibited the PDGF-BB- and -AA-induced growth but had no effect on EGF- or FGF-2-induced growth (Figure 2). In an additional experiment we also documented a 84% inhibition of the mesangial cell growth induced by 50 ng/ml PDGF-BB at an aptamer concentration of 0.5 µg/ml, ie, the minimal aptamer level achieved *in vivo* (see above). Inhibition of PDGF-BB-induced growth by the PDGF-B aptamer was not due to cell death as evidenced by trypan blue exclusion of the cells. Furthermore, using morphological criteria, in particular, nuclear condensation, no evidence for mesangial cell apoptosis was noted under these conditions.

Using the XTT assay (Table 1), the PDGF-B aptamer also completely inhibited PDGF-BB-induced human mesangial cell growth. PDGF-AB- and -AA-induced mesangial cell growth also tended to be lower with the PDGF-B aptamer, but these differences failed to reach statistical significance. In contrast, no effects of the PDGF-B aptamer on either EGF- or FGF-2-induced growth were noted (Table 1). Similar effects were noted if the aptam-

Table 1. Effects of PDGF-B Aptamer on Mitogen-Stimulated Proliferation of Human Mesangial Cells in Culture

	Medium	PDGF-BB	PDGF-AB	PDGF-AA	EGF	FGF-2
PDGF-B aptamer (50 μg/ml)	98 ± 23	108 ± 15*	76 ± 34	100 ± 29	159 ± 14	162 ± 20
Scrambled aptamer (50 μg/ml)	88 ± 19	228 ± 65	123 ± 36	142 ± 41	160 ± 21	163 ± 38
40-kd PEG alone	100 ± 0	218 ± 92	159 ± 40	142 ± 56	155 ± 33	174 ± 22

All mitogens were added at 100 ng/ml final concentration. Data are optical densities measured in the XTT assay and are expressed as percentages of baseline, ie, cells stimulated with medium plus 200 μg/ml 40-kd PEG (ie, the amount equivalent to the PEG attached to 50 μg/ml aptamer). Results are means ± SD of five separate experiments (n = 3 in the case of medium plus 40-kd PEG; statistical evaluation was therefore confined to the PDGF-B and scrambled aptamer groups.
*$P < 0.05$ *versus* scrambled aptamer.

ers were used at a concentration of 10 μg/ml (data not shown).

Effects of PDGF B-Chain DNA-Aptamer in Rats with Anti-Thy-1.1 Nephritis

After the injection of anti-Thy-1.1 antibody, PBS-treated animals developed the typical course of the nephritis, which is characterized by early mesangiolysis and followed by a phase of mesangial cell proliferation and matrix accumulation on days 6 and 9. No obvious adverse effects were noted after the repeated injection of aptamers or PEG alone, and all rats survived and appeared normal until the end of the study.

In PAS-stained renal sections the mesangioproliferative changes on days 6 and 9 after disease induction were severe and indistinguishable among rats receiving PBS, PEG alone, or the scrambled aptamer (Figure 3, A to C). Histological changes were markedly reduced in the PDGF-B aptamer-treated group (Figure 3D). To evaluate the mesangioproliferative changes, various parameters were analyzed.

Reduction of Mesangial Cell Proliferation

Glomerular cell proliferation, as assessed by counting the number of glomerular mitoses, was not significantly different between the three control groups on days 6 and 9 (Figure 4). Compared with rats receiving the scrambled aptamer, treatment with PDGF-B aptamer led to a reduction of glomerular mitoses by 64% on day 6 and by 78% on day 9 (Figure 4). To assess the treatment

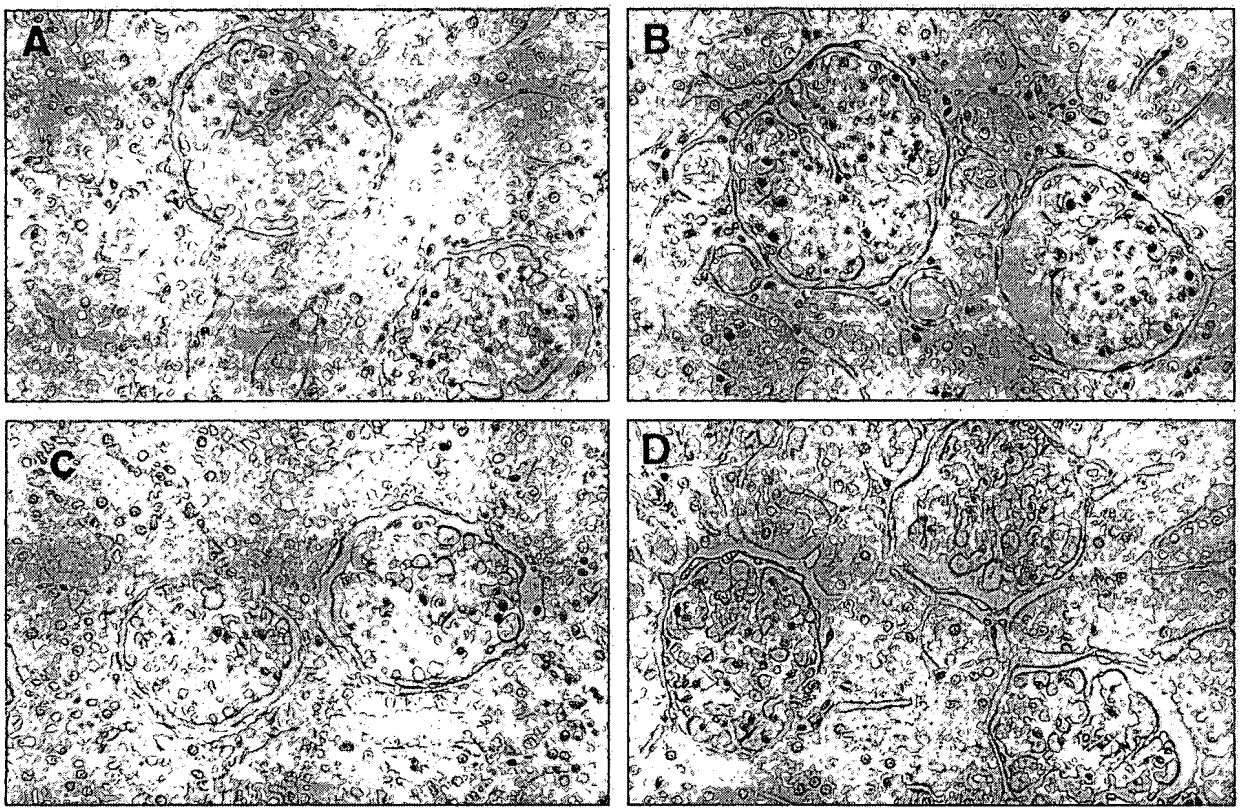

Figure 3. Representative PAS-stained renal sections at day 9 after disease induction of mesangioproliferative nephritis of a rat receiving PBS alone (A), 40-kd PEG alone (B), scrambled aptamer coupled to 40-kd PEG (C), or PDGF-B aptamer coupled to 40-kd PEG (D). Compared with the three control rats shown in A to C, the PDGF-B aptamer-treated rat exhibits an almost normal glomerular morphology, ie, markedly reduced glomerular hypercellularity and mesangial matrix accumulation. Magnification, ×400.

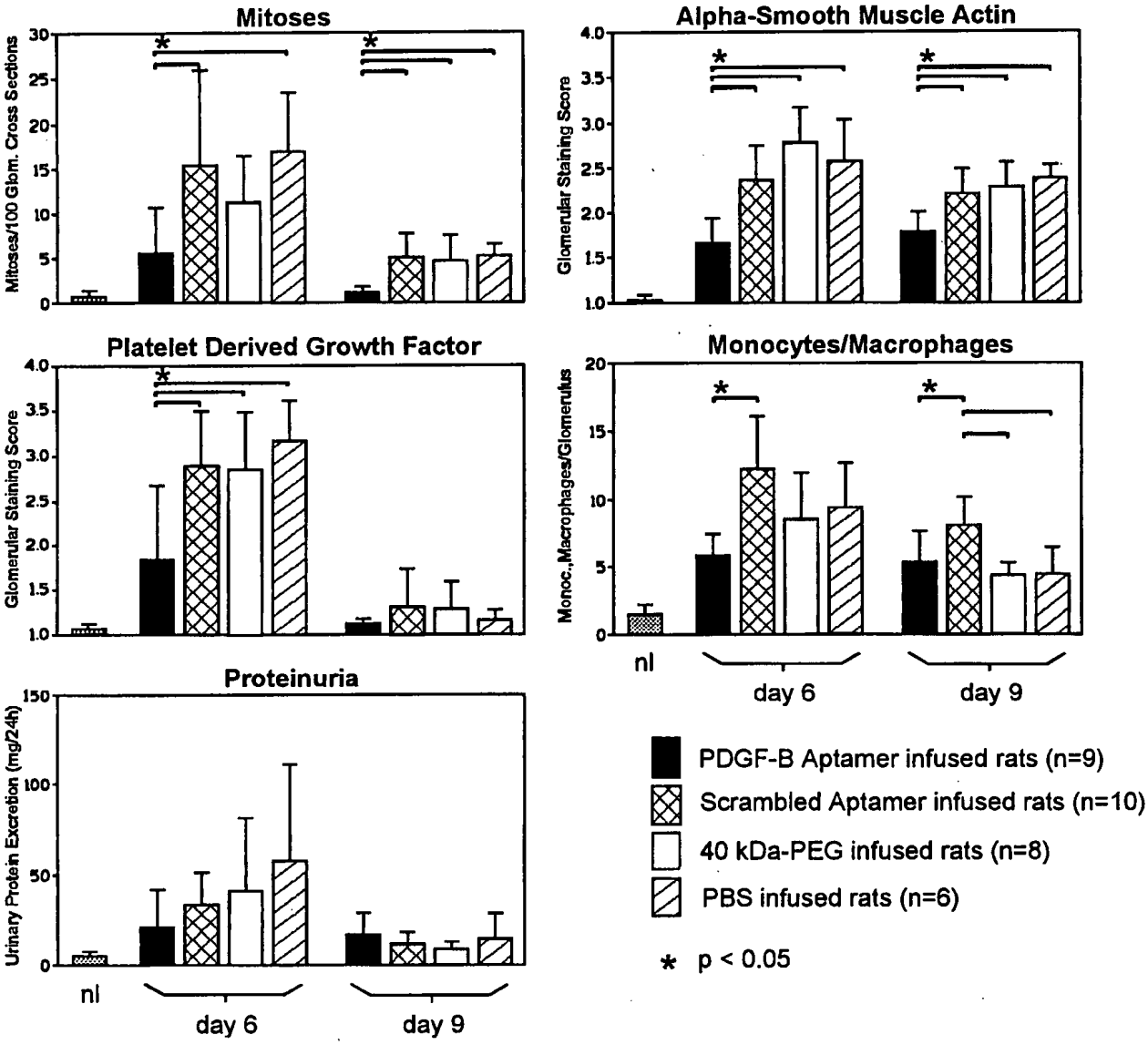

Figure 4. Effects of PDGF-B aptamer on glomerular cell proliferation, mesangial cell activation (as assessed by glomerular *de novo* expression of α-smooth muscle actin), expression of glomerular PDGF B-chain, monocyte/macrophage influx, and proteinuria in rats with anti-Thy-1.1 nephritis. nl = values observed in 10 normal rats.

effects on mesangial cells, we immunostained the renal sections for α-smooth muscle actin, which is expressed by activated mesangial cells only.[24] Again, there were no significant differences between the three control groups on days 6 and 9. However, the immunostaining scores of α-smooth muscle actin were significantly reduced on days 6 and 9 in the PDGF-B aptamer-treated group (Figure 4). To specifically determine whether mesangial cell proliferation was reduced, we double immunostained PDGF-B aptamer-treated rats and scrambled aptamer-treated rats for a cell proliferation marker (BrdU) and α-smooth muscle actin. The data confirmed a marked decrease of proliferating mesangial cells on day 9 after disease induction: 2.2 ± 0.8 BrdU-positive/α-smooth-muscle-actin-positive cells per 100 glomerular cross sections in PDGF-B aptamer-treated rats *versus* 43.3 ± 12.4 cells in rats receiving the scrambled aptamer, ie, a 95% reduction of mesangial cell proliferation (Figure 5). In contrast, no effect of the PDGF-B aptamer was noted on proliferating monocytes/macrophages on day 9 after disease induction (PDGF-B aptamer-treated rats: 2.8 ± 1.1 $BrdU^+/ED\text{-}1^+$ cells per 100 glomerular cross sections; scrambled aptamer-treated rats: 2.7 ± 1.8).

Reduced Expression of Endogenous PDGF B-Chain

By immunohistochemistry the glomerular PDGF B-chain expression was markedly up-regulated in all three control groups (Figure 4), similar to previous observations.[15] In the PDGF-B aptamer-treated group the glomerular overexpression of PDGF B-chain was significantly reduced in parallel with the reduction of proliferating mesangial cells (Figure 4). This reduction

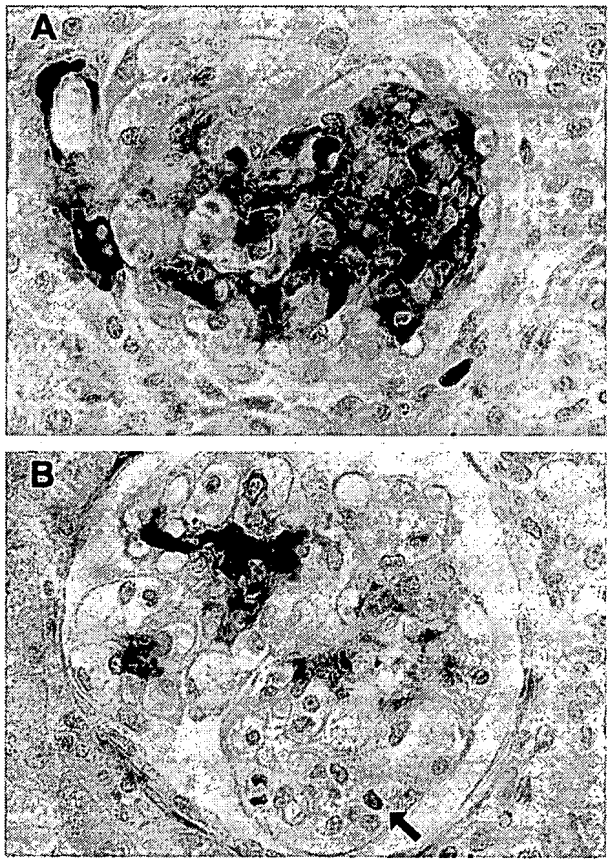

Figure 5. Effects of PDGF-B aptamer on glomerular mesangial cell proliferation as assessed by double immunostaining for nuclei incorporating the thymidine analogue BrdU and the mesangial cell *de novo* expression of α-smooth muscle actin on day 9 after disease induction. In a rat receiving scrambled DNA aptamer (A), marked glomerular *de novo* expression of α-smooth muscle actin (red stain) is noted, and at least three glomerular cells with a BrdU-positive nucleus (brown stain) exhibit an α-smooth muscle actin-positive cytoplasm, indicating that they are proliferating mesangial cells. In a rat receiving PDGF-B aptamer (B), little glomerular *de novo* expression of α-smooth muscle actin (red stain) is noted, and only one glomerular cell, which is α-smooth muscle actin negative, shows weak nuclear BrdU staining (arrow). Magnification, ×400.

was not due to masking of the PDGF B-chain epitope recognized by the anti-PDGF B-chain antibody, as the immunostaining intensity was not affected in renal sections that had been preincubated with the PDGF-B aptamer (immunostaining scores in sections preincubated with buffer, 2.70; with PDGF-B aptamer, 2.97; with scrambled aptamer, 2.78; means of two experiments each).

Reduction of Glomerular Monocyte/Macrophage Influx

The glomerular monocyte/macrophage influx was significantly reduced in the PDGF aptamer-treated rats as compared with rats receiving scrambled aptamer on days 6 and 9 after disease induction (Figure 4).

Effects on Proteinuria

Moderate proteinuria of up to 147 mg/24 hours was present on day 6 after disease induction in the three control groups (Figure 4). Treatment with the PDGF-B aptamer reduced the mean proteinuria on day 6, but this failed to reach statistical significance (Figure 4). Proteinuria on day 9 after disease induction was low and similar in all four groups (Figure 4).

Reduction of Glomerular Matrix Production and Accumulation

By immunohistochemistry, marked glomerular accumulation of type IV collagen and fibronectin was noted in all three control groups (Figure 6). The overexpression of both glomerular type IV collagen and fibronectin was significantly reduced in PDGF-B aptamer-treated rats (Figure 6). By *in situ* hybridization, the decreased glomerular protein expression of type IV collagen in PDGF-B aptamer-treated rats was shown to be associated with decreased glomerular synthesis of this collagen type (Figure 6). Sense controls for the *in situ* hybridizations were negative and similar to those published recently[25] (Figure 6E).

Discussion

In the anti-Thy-1.1 nephritis model, immunological damage to the mesangium, resulting in mesangiolysis, occurs during the first 24 to 48 hours after disease induction.[13,26] Subsequently, nondamaged mesangial cells immigrate from the extraglomerular mesangium and proliferate, giving rise to the pathological picture of mesangioproliferative glomerulonephritis.[7,27] Given this course of the nephritis, we initiated treatment on the 3rd day to avoid any interference of the therapy with pathomechanisms involved in disease induction, eg, glomerular binding of the nephritogenic antibody, complement activation, or cytotoxic damage, all of which peak during the 1st day of the disease. Furthermore, this experimental design enhances the clinical relevance of the study, as treatment was instituted after the onset of mesangioproliferative glomerulonephritis.

The major finding of the present study was that treatment with PDGF-B aptamer from days 3 to 9 after disease induction resulted in a near complete arrest of the overshooting mesangial cell proliferation and thereby led to a marked reduction of glomerular hypercellularity. Further support for the reduction of mesangial cell proliferation is provided by the observations that the glomerular *de novo* expression of α-smooth muscle actin and the overexpression of PDGF B-chain were also significantly inhibited by the PDGF-B aptamer. Both of these proteins are selectively up-regulated in activated mesangial cells in the anti-Thy-1.1 model,[15,24] and consequently, any intervention that reduces mesangial cell hyperplasia should also reduce expression of these proteins. It is of interest to note that mesangial cell proliferation as assessed by double immunostaining for BrdU and α-smooth muscle actin expression was reduced by 95% although glomerular mitoses were reduced by only 78% on day 9. This discrepancy suggests that proliferation of other glomer-

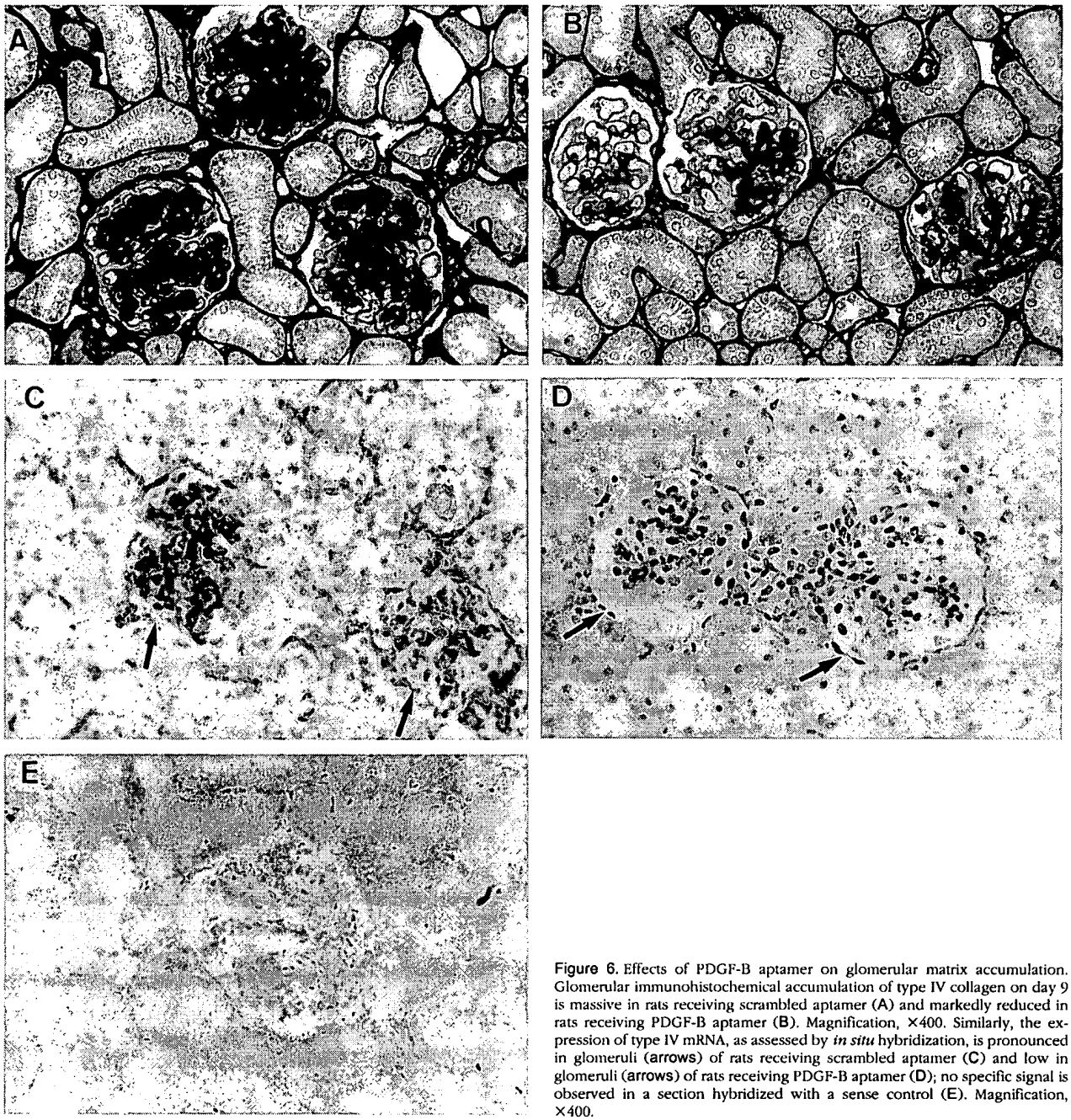

Figure 6. Effects of PDGF-B aptamer on glomerular matrix accumulation. Glomerular immunohistochemical accumulation of type IV collagen on day 9 is massive in rats receiving scrambled aptamer (**A**) and markedly reduced in rats receiving PDGF-B aptamer (**B**). Magnification, ×400. Similarly, the expression of type IV mRNA, as assessed by *in situ* hybridization, is pronounced in glomeruli (**arrows**) of rats receiving scrambled aptamer (**C**) and low in glomeruli (**arrows**) of rats receiving PDGF-B aptamer (**D**); no specific signal is observed in a section hybridized with a sense control (**E**). Magnification, ×400.

ular cells may be independent of PDGF (as demonstrated, for example, by the determination of proliferating monocytes/macrophages) and lends support to the highly specific action of the PDGF-B aptamer.

In addition to selectively abrogating mesangial cell proliferation, treatment with the PDGF-B aptamer also reduced the glomerular monocyte/macrophage influx and accumulation of extracellular matrix. Both of these latter processes are known to depend to a large degree on other cytokines produced by activated mesangial cells: glomerular macrophage influx on the production of monocyte chemoattractant protein-1[28] and matrix overproduction on the release of transforming growth factor-β.[29] The present data therefore suggest that PDGF B-chain is either linked to the mesangial cell production of these latter cytokines, as indicated by cell culture studies,[30] or that PDGF B-chain is directly and independently involved in chemotaxis and matrix production. Regardless of these issues, the present study establishes the release of PDGF B-chain as a central pathogenetic event in the development of mesangioproliferative glomerulonephritis. Our data also suggest that macrophage- or platelet-derived PDGF is unlikely to play a major role in the mediation of mesangioproliferative changes in this model, as influx of both cell types peaks at ~24 hours,[13] ie, 2 days before the initiation of anti-PDGF treatment.

Similar, albeit quantitatively somewhat lower, effects were also noted in our previous study with a neutralizing antibody to PDGF B-chain in the anti-Thy-1.1 nephritis model; treatment resulted in a 57% reduction of glomerular cell proliferation on day 4 and reduced glomerular matrix accumulation by approximately one score point.[6] One important concern in that study was that large amounts of IgG (600 mg/kg/day), both neutralizing anti-PDGF antibody and control IgG, had to be administered on a daily basis to the rats. Large doses of IgG can ameliorate the course of immune-mediated renal disease,[31] and the therapeutic effect of IgG *per se* is variable from batch to batch (R. J. Johnson and J. Floege, personal observation). Second, mesangial cells express Fc-γ receptors and modulate their cytokine release on binding of IgG to Fc receptors.[10] These observations, in addition to the immunogenicity of heterologous antibodies, impose considerable restrictions on the interpretation of such studies.

At present, no other specific PDGF B-chain antagonists have been tested *in vivo*. Trapidil, an anti-platelet agent, has been shown to interfere with PDGF B-chain binding to mesangial PDGF receptors, but its action is not specific for PDGF.[32] Preliminary data suggest that a blocker of the PDGF receptor-associated tyrosine kinase may also ameliorate the course of mesangioproliferative nephritis,[33] but again, the compound was not specific for PDGF and also reduced, for example, basic-FGF-induced cell proliferation.

The possibility to develop aptamer antagonists for defined biological mediators has become an attractive novel therapeutic approach in various diseases.[23] These previous studies, in which thrombin, selectins, and neutrophil elastase were targeted, have demonstrated the ability of aptamers to act as antagonists of plasma or leukocyte proteins *in vivo*.[34–36] The present study is the first to demonstrate that specific antagonism of peptide growth factors, which are produced in a spatially much more confined manner, by high-affinity aptamers *in vivo* is not only feasible but also highly effective. In pilot studies the high effectiveness of the PDGF-B aptamer was also demonstrated in an experiment in which the injection of 5 mg of the aptamer at a single time point, ie, day 3 of the nephritis, was as efficient as several days of twice-daily treatment with a total of 10 to 30 mg (unpublished data). Furthermore, in pilot experiments we have observed that the effects of the PDGF-B aptamer *in vivo* are dose dependent and can be demonstrated with doses as low as 2 mg total. In support of our findings, the PDGF aptamer used in this study has recently exhibited efficacy in the rat model of restenosis (C.-H. Heldin and A. Östman, personal communication).

Apart from their specificity and potency, the use of aptamers largely circumvents problems of Fc receptor binding. Furthermore, although the effort to thoroughly examine the immunogenicity of aptamers is still in progress, extensive *in vivo* data obtained to date suggest that aptamers induce little if any immune response (J. Bill and G. Biesecker, unpublished results). Another attractive feature of aptamers is that their pharmacokinetic behavior can easily be altered, for example, by altering the molecular size (eg, via variation of the length of the attached PEG), hydrophobicity (eg, via packaging them in or on liposomes[9]), or other features. A potential problem with the use of nucleic-acid-based antagonists is their polyanionic character, which might in itself affect the course of diseases. Thus, other polyanionic compounds, for example, heparins or heparan sulfate proteoglycans, are also effective in reducing mesangial cell proliferation in the anti-Thy-1.1 nephritis model.[14] However, in the present study, this concern was resolved by the ineffectiveness of a scrambled aptamer, which is identical in composition and predicted secondary structure to the PDGF-B aptamer but whose binding affinity for PDGF B-chain is dramatically lower.

In conclusion, we describe the efficacy of a high-affinity nucleic-acid-based growth factor antagonist *in vivo* and thereby extend the currently available evidence that PDGF B-chain is a central mediator in mesangioproliferative glomerulonephritis. The specificity and effectiveness demonstrated with the currently used aptamer opens the possibility for growth factor inhibition over prolonged periods. Aptamers may thus be used both to elucidate the role of individual growth factors that represent potential targets for therapeutic intervention and to serve as drug candidates for the treatment of disease states characterized by growth factor overexpression.

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Expression of a Novel PDGF Isoform, PDGF-C, in Normal and Diseased Rat Kidney

FRANK EITNER,* TAMMO OSTENDORF,* CLAUDIA VAN ROEYEN,* MASASHI KITAHARA,* XURI LI,† KARIN AASE,† HERMANN-JOSEF GRÖNE,‡ ULF ERIKSSON,‡ and JÜRGEN FLOEGE*

**Division of Nephrology and Immunology, University of Aachen, Aachen, Germany; †Ludwig Institute for Cancer Research, Stockholm, Sweden; and ‡German Cancer Research Institute, DKFZ Heidelberg, Germany.*

Abstract. Platelet-derived growth factor–C (PDGF-C) is a new member of the PDGF family. Its expression in normal and diseased kidney is unknown. Rabbit antisera were generated against human full-length, core domain, and mouse PDGF-C, and their specificity was confirmed by Western blot analyses. Renal PDGF-C expression was analyzed by immunohistochemistry in normal rats (n = 8), mesangioproliferative anti-Thy 1.1 nephritis (n = 4 each at days 1, 4, 6, and 85), passive Heymann nephritis (PHN, n = 4), puromycin nephrosis (PAN, n = 2), Milan normotensive rats (MN, n = 2), and obese Zucker rats (n = 3). PDGF-C expression was also studied in anti-Thy 1.1 rats treated with PDGF-B aptamer antagonists (n = 5) or irrelevant control aptamers (n = 5). PDGF-C was constitutively expressed in arterial smooth muscle cells and collecting duct epithelial cells. Mesangial PDGF-C was markedly upregulated in anti-Thy 1.1 nephritis in parallel with the peak mesangial cell proliferation. Furthermore, PDGF-CC acted as a potent growth factor for mesangial cells *in vitro*. Inhibition of PDGF-B via specific aptamers reduced the injury in anti-Thy 1.1 nephritis but did not affect the glomerular PDGF-C overexpression or the mitogenicity of PDGF-CC *in vitro*. In PHN, PAN, and obese Zucker rats, glomeruli remained negative for PDGF-C despite severe glomerular injury. PDGF-C localized to podocytes at sites of focal and segmental sclerosis in MN. Interstitial PDGF-C expression was increased at sites of fibrosing injury in obese Zucker rats. The use of the different antisera resulted in virtually identical findings. It is concluded that PDGF-C is a novel mesangial cell mitogen that is constitutively expressed in the kidney and specifically upregulated in mesangial, visceral epithelial, and interstitial cells after predominant injury to these cells. PDGF-C may therefore be involved in the pathogenesis of renal scarring.

Members of the platelet-derived growth factor (PDGF) family of cytokines are important mitogens and chemoattractants for many types of mesenchymal cells (1–3). Until recently, the PDGF family comprised three dimers composed of a PDGF A- and B-chain, *i.e.* PDGF-AA, -AB, and -BB (4). It is well established by now that these PDGF are involved in different aspects of renal disease, in particular the mediation of glomerular mesangial cell proliferation and the induction of renal interstitial fibrosis (5,6). Both known subunits of the PDGF receptor, *i.e.* the α-subunit and β-subunit are constitutively expressed in the kidney. Although the α-subunit is present in vascular smooth muscle cells and the renal interstitium, the β-subunit is constitutively expressed in mesangial and parietal glomerular epithelial cells, in vascular smooth muscle cells, and in renal interstitial cells (4). Increased expression of PDGF receptors at sites of renal injury has been documented in a large variety of diseases (4).

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Correspondence to: Dr. Frank Eitner, Klinikum der RWTH Aachen, Medizinische Klinik II (Nephrologie und Immunologie), Pauwelsstr 30, 52074 Aachen, Germany. Phone: 49-241-8089-670; Fax: 49-241-8082-446; E-mail: feitner@ukaachen.de

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A new member of the PDGF family has recently been identified and subsequently termed PDGF-C (7). PDGF-C, like PDGF-A and -B, forms a disulphide-bonded dimer, PDGF-CC. Li *et al.* (7) identified PDGF-CC as a PDGFR-α–specific ligand, and Gilbertsson *et al.* (8) showed that PDGF-CC could activate the beta receptor in a heterodimeric complex. There is no detectable binding or activation to beta receptor homodimers (Gilbertsson *et al.* (8) and our unpublished data). Transgenic overexpression of PDGF-C in the heart induced a significant proliferation of myocardial interstitial cells in addition to an increase in extracellular matrix production (7). To date, information on PDGF-C in the kidney is limited to the demonstration of abundant PDGF-C transcripts in human kidney (7). In addition, strong PDGF-C mRNA expression was detected in the metanephric mesenchymal aggregates during murine nephrogenesis, suggesting a role for PDGF-C in mesenchymal epithelial conversion as a prelude to tubular development (7). This study is the first to identify PDGF-C as a potent mitogenic stimulus for cultured mesangial cells *in vitro*. The localization of the cytokine PDGF-C in normal or diseased adult renal tissues as well as its function *in vivo* are unknown. We therefore analyzed the expression of PDGF-C by immunohistochemistry in renal tissues obtained from healthy normal adult rats and from rats with different renal diseases. Studied renal diseases included rats with immune-mediated mesangioproliferative anti-Thy 1.1 glomerulonephritis, rats with im-

mune-mediated podocyte injury (passive Heymann nephritis [PHN]), rats with toxic podocyte injury (puromycine aminonucleoside nephrosis [PAN]), rats developing a spontaneous glomerulosclerosis (Milan normotensive rats), and obese Zucker rats with hyperlipidemic and type II diabetic renal damage. Although PDGF-B is a known potent mesangial mitogen *in vivo*, a potential role of PDGF-C in mesangioproliferative glomerulonephritis *in vivo* is currently unknown. To address whether PDGF-C, which at least partially binds to the same receptors as PDGF-B, uses different pathways than PDGF-B in mediating mesangial cell proliferation *in vivo*, we also analyzed whether inhibition of PDGF-B (via PDGF-B specific aptamers) influenced the expression of PDGF-C in the anti-Thy 1.1 glomerulonephritis model.

Materials and Methods

Animal Models

All animal studies were approved by the local Institutional Review Board.

Normal Rats. Eight male Wistar rats (Charles River Wiga GmbH, Sulzfeld, Germany) weighing 140 to 180 g remained untreated and served as healthy control animals.

Mesangioproliferative Anti-Thy 1.1 Nephritis. Anti-Thy 1.1 nephritis was induced in 16 male Wistar rats (Charles River Wiga GmbH; weighing 160 to 180 g at the start of the experiment) by intravenous injection of 1 mg/kg monoclonal anti-Thy 1.1 antibody (clone OX-7; European Collection of Animal Cell Cultures, Salisbury, UK) as described (9). Animals remained untreated and were sacrificed at days 1, 4, 6, and 85 after induction of the disease ($n = 4$ at each time point).

Antagonism of PDGF-B after Induction of Anti-Thy 1.1 Glomerulonephritis. PDGF-C expression was additionally studied in ten rats that were treated with PDGF-B–specific or irrelevant (scrambled) control aptamers as described (9). Anti-Thy-1.1 nephritis was induced in ten male Wistar rats (Charles River Wiga GmbH) as described above. Rats were treated with aptamers from days 3 until sacrifice at day 6 after disease induction. Treatment consisted of twice-daily intravenous bolus injections of the substances dissolved in 400 μl of phosphate-buffered saline (PBS), pH 7.4. Five rats received 0.66 mg/d PDGF-B specific aptamer, and five rats received 0.66 mg/d scrambled aptamer.

Passive Heymann Nephritis. PHN was induced in four male Sprague-Dawley rats (Charles River Wiga GmbH; weighing 230 to 240 g) by intravenous injection of 0.8 ml of sheep anti-Fx1a antibody per rat (10). Animals remained untreated and were sacrificed at day 8 after induction of the disease.

Puromycine Aminonucleoside Nephrosis. PAN was induced in two male Sprague-Dawley rats (Charles River Wiga GmbH; weighing 210 to 230 g) by intravenous injection of 150 mg/kg puromycin (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) dissolved in normal saline as described (11). Renal tissues were obtained after sacrifice on day 7.

Milan Normotensive Rats. Two Milan normotensive rats were obtained from the Hannover Medical School, Germany. Renal tissues were obtained after sacrifice at 40 wk of age (12).

Obese Zucker Rats. Three male obese (*fa/fa*) Zucker rats were obtained from Charles River Wiga. Renal tissues were obtained after sacrifice at 60 wk of age (13).

Tissues for morphologic evaluation and immunohistochemical analyses were fixed in methyl Carnoy solution, embedded in paraffin, and sectioned. The presence of morphologic features of the different renal diseases was examined in periodic acid-Schiff–stained (PAS-stained) sections. All tissue sections contained a minimum of 50 (usually >100) glomerular cross-sections.

Antibodies

Rabbit antisera directed against PDGF-C were generated as described previously in detail (7). Three different antisera preparations were used in the study: (*1*) anti-human full-length PDGF-CC, affinity-purified against the core domain of PDGF-CC; (*2*) anti-human PDGF-CC core domain, affinity-purified against the core domain of PDGF-CC; and (*3*) anti-mouse PDGF-CC peptide, affinity-purified against the core domain of PDGF-CC. Specificity of these antisera for the detection of PDGF-C has been demonstrated previously by Western blot analyses (7). Further analysis of the specificity of the antisera in immunohistochemical procedures was performed as part of the present study and is detailed below.

Rat renal collecting duct epithelial cells were detected with a rabbit polyclonal antibody directed against rat aquaporin-2 (14). Aquaporin-2 is a vasopressin-regulated water channel expressed exclusively in the renal collecting duct. The antibody was a kind gift of Dr. Mark Knepper, Renal Mechanisms Section, NHLBI, NIH, Bethesda, MD, USA. Additional primary antibodies were identical to those described previously (15,16) and included a murine monoclonal antibody (clone 1A4) to smooth muscle actin, a murine monoclonal antibody (clone PGF-007) to PDGF B-chain, a murine monoclonal IgG antibody (clone ED1) to a cytoplasmic antigen present in monocytes, macrophages, and dendritic cells, affinity-purified polyclonal goat anti-human/bovine type IV collagen IgG preabsorbed with rat erythrocytes, an affinity-purified IgG fraction of a polyclonal rabbit anti-rat fibronectin antibody, and appropriate negative controls as described previously (15,16).

Immunohistochemical Analyses

Immunohistochemical analyses were performed following previously published protocols (17,18). Briefly, methyl Carnoy-fixed, paraffin-embedded tissues were sectioned at 4 μ. Sections were deparaffinized in xylene and rehydrated in graded ethanols. Endogenous peroxidase was blocked by incubation in 3% hydrogen peroxide. The sections were then incubated for 1 h with the primary antibody diluted in PBS containing 1% bovine serum albumin (Sigma). After washes in PBS, the sections were sequentially incubated with biotinylated goat anti-rabbit antibody (Vector, Burlingame, CA), the ABC-Elite reagent (Vector), and finally 3,3'-diaminobenzidine (DAB, Sigma) with nickel chloride enhancement used as the chromogen. Sections were counterstained with methyl green, mounted, and coverslipped. Negative controls consisted of replacement of the primary antiserum with nonimmune rabbit serum. In pilot experiments, we tested the sensitivity and specificity of our procedure. The antibodies were tested at final concentrations between 1 and 15 μg/ml. For the evaluation of the immunoperoxidase stains for α–smooth muscle actin, PDGF-B, and PDGF-C, each glomerular area was graded semiquantitatively, and the mean score per biopsy was calculated. Each score mainly reflects changes in the extent rather than intensity of staining and depends on the percentage of the glomerular tuft area showing focally enhanced positive staining: I, 0% to 25%; II, 25% to 50%; III, 50% to 75%; IV, >75%. We have recently described that data obtained using this scoring system are highly correlated with those obtained by computerized morphometry (19).

Cell Cultures/Mesangial Cell Proliferation Assay

The generation of primary rat mesangial cells has been previously described in detail (20). Cells were grown in RPMI 1640 (Sigma) supplemented with 15% fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were cultured at 37°C in 5% CO_2 and were passaged at subconfluency by harvesting with trypsin/ethylenediaminetetraacetic acid. The incorporation of 5-bromo-2'-deoxyuridine (BrdU) into DNA was used as a measurement of mesangial cell proliferation. Mesangial cells (3×10^3 cells/well) were transferred to a 96-well microtiter plate and grown in RPMI 1640 containing 15% FCS until the cells were subconfluent. After incubation for 24 h in RPMI 1640 with 0.5% FCS, cells were stimulated for another 24 h with purified PDGF-CC protein (1 to 50 ng/ml [7]), PDGF-BB protein (1 to 50 ng/ml), PDGF-AA protein (1 to 50 ng/ml) (PDGF-AA and PDGF-BB were kindly provided by J. Hoppe, University of Würzburg, Germany). Cells were labeled with BrdU during the last 4 h of culture according to the manufacturers instructions (Cell proliferation enzyme-linked immunosorbent assay [ELISA], Roche Diagnostics GmbH, Mannheim, Germany). At the end of the incubation period, adherent mesangial cells were washed and denatured. Incorporated BrdU was detected by using a peroxidase-labeled anti-BrdU antibody and a peroxidase color substrate. Finally, the absorbance of the samples was measured in an ELISA reader at 370 nm. Cell proliferation experiments were independently performed four times with duplicate measurements.

Western Blot Analyses

PDGF-C expression was additionally analyzed in protein lysates from cultured primary rat mesangial cells from primary rat smooth muscle cells and from isolated glomerular fractions as described (21). Briefly, cells or isolated glomeruli were homogenized in Triton X-100 lysis buffer (50 mM Hepes, pH 7.5; 150 mM NaCl; 1.5 mM $MgCl_2$; 1 mM ethyleneglycotetraacetic acid; 10% glycerol; 1% Triton X-100; 1 μg/ml aprotinin; 1 μg/ml leupeptin; 1 mM phenylmethylsulfonyl chloride; 0.1 mM sodium orthovanadate) at 4°C. Incubation for 5 min preceded ultrasound treatment (3×10 s). The protein concentrations of the resulting solutions were determined using the BCA protein assay (Pierce, Rockford, IL). Forty micrograms of protein were electrophoresed under reducing conditions on a 10% sodium dodecyl sulfate gel and then blotted onto nitrocellulose membranes. The blots were blocked with 2% bovine serum albumin (Sigma) in TTBS (150 mM NaCl; 10 mM Tris, pH 8.0; 0.05% (vol/vol) Tween 20) for 1 h at room temperature and then incubated with the anti-human PDGF-CC core domain antibody diluted in TTBS overnight at 4°C. After several washes in TTBS, the blots were incubated with peroxidase-conjugated horse anti-rabbit IgG antibody (Vector) for 1 h. The blots were visualized with the enhanced chemiluminescence reagent (ECL; Amersham Pharmacia Biotech, Freiburg, Germany).

Statistical Analyses

Data are given as mean ± SD. Statistical significance, defined as $P < 0.05$, was evaluated by using the t test.

Results

PDGF-C Expression in Normal Adult Rat Kidney

The immunohistochemical pattern of PDGF-C expression demonstrated a very consistent result in all eight analyzed animals (Table 1). The majority of glomeruli of normal rats were completely negative for PDGF-C (Figure 1A). Very few glomeruli of each case showed some weak granular PDGF-C immunohistochemical signal. However, this weak staining could not be clearly attributed to a specific glomerular cell type. Parietal epithelial cells were negative for PDGF-C. PDGF-C expression was regularly identified in the vascular compartment. Smooth muscle cells of arteries and arterioles constitutively expressed PDGF-C (Figure 1C). The immunohistochemical signal localized within the cytoplasm of smooth muscle cells and occasionally appeared to reflect a granular

Table 1. PDGF-C protein expression in normal and diseased adult rat kidneys[a]

	Normal	Thy 1.1	PAN	PHN	Milan	*fa/fa*
Glomeruli						
mesangial cells	−	++	+/−	(+/−)	−	−
endothelial cells	−	−	−	−	−	−
visceral epithelial cells	−	−	−	−	+/−	−
parietal epithelial cells	−	−	−	+/−	+/−	−
Vessels						
endothelial cells	−	−	−	−	−	−
smooth muscle cells	+	+	+	+	+	+
adventitial cells	−	−	−	−	−	−
Tubulointerstitium						
proximal tubules	−	−	−	−	−	−
distal tubules	−	−	−	−	−	−
loop of Henle	−	−	−	−	−	−
collecting ducts	+	+	+	+	+	+
interstitial cells	+/−	−	−	+/−	−	+

[a] Normal, normal adult rat; Thy 1.1, mesangioproliferative anti-Thy 1.1. glomerulonephritis, day 6; PAN, puromycin aminonucleoside nephrosis; PHN, passive Heymann nephritis; Milan, Milan normotensive glomerulosclerosis; *fa/fa*, obese (fatty) Zucker rats. For details see Materials and Methods and Results sections.

−, no expression detectable; +/−, weak and/or variable expression; +, detectable expression.

deposition within the cells. PDGF-C expression remained undetectable in endothelial cells of all analyzed cases. Within the tubulointerstitial compartment, PDGF-C expression localized to a limited number of tubular structures (Figure 1E). Serial sections labeled with an antibody directed against aquaporin-2 clearly identified the PDGF-C–expressing tubular segments as collecting ducts (Figure 1F). Both cortical and outer medullary collecting ducts expressed PDGF-C. Proximal as well as distal tubular epithelial cells were negative for PDGF-C. Negative control tissue sections that were incubated with equal amounts of nonimmune rabbit serum did not demonstrate any staining signal in all analyzed normal and diseased cases.

Mesangial PDGF-C is Markedly Upregulated during Experimental Mesangioproliferative Glomerulonephritis

The anti-Thy 1.1 glomerulonephritis is a model of an antibody-induced, complement-mediated mesangiolysis, followed by a phase of mesangial cell proliferation with peak proliferation between 4 and 6 d after disease induction, and finally spontaneous restitution of normal glomerular morphology within 2 to 3 mo. Typical histologic features of this disease model were observed in the 16 animals analyzed in the present study. Glomerular PDGF-C expression was markedly altered in the course of the mesangioproliferative glomerulonephritis as compared with normal rats. By Western blot analyses, an upregulated glomerular PDGF-C expression was identified in the course of the glomerulonephritis (Figure 2). Although PDGF-C was undetectable in protein lysates from isolated glomeruli obtained from normal rats, an intense band corresponding with the proteolytically activated 33-kD PDGF-C protein could be identified in glomeruli obtained from rats at day 6 after anti-Thy 1.1 disease induction. This result was confirmed and further refined by our immunohistochemical studies (Table 1). Very early, at day 1 following disease induction, a weak granular immunohistochemical signal was observed in most glomeruli. The granular signal was detectable in a mesangial distribution pattern. In parallel to the peak mesangial cell activation/proliferation at days 4 and 6, there was a marked increase in mesangial PDGF-C expression (Figure 3). The immunohistochemical signal specifically localized to the mesangium, and glomerular endothelial cells and visceral epithelial cells remained negative for PDGF-C (Figure 3). In addition to the prominent mesangial expression, PDGF-C was detected in parietal epithelial cells of individual glomeruli at day 6 (data not shown). Circulating leukocytes within glomerular microaneurysms typically present at days 4 and 6 represented another frequent localization of glomerular PDGF-C. With the resolution of the glomerular injury at day 85, the majority of glomeruli became negative for PDGF-C. A limited number of glomeruli at day 85 continued to demon-

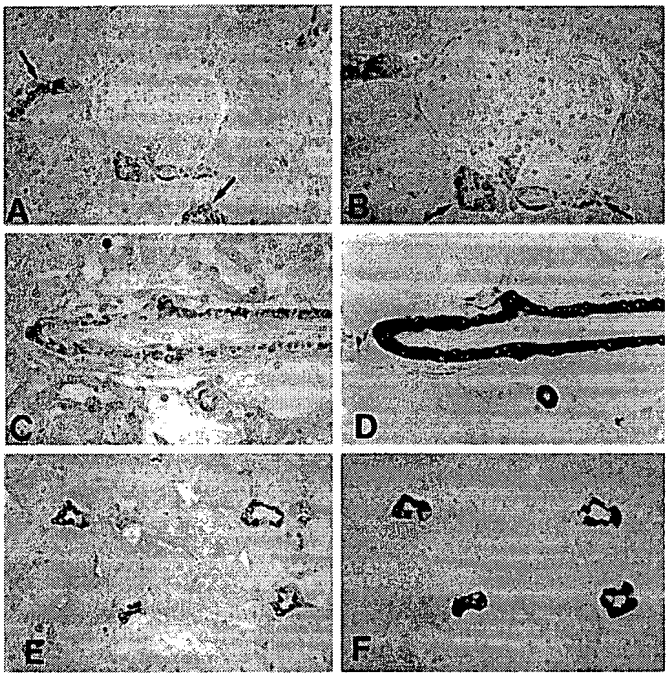

Figure 1. Platelet-derived growth factor–C (PDGF-C) protein expression in normal adult rat kidney. (A) In the normal rat kidney, PDGF-C is expressed in arteriolar smooth muscle cells and in epithelial cells of individual tubular segments (arrows). (B) Higher power magnification illustration of the same glomerulus as illustrated in panel A, showing PDGF-C localized to smooth muscle cells of afferent and efferent arteriole (arrows). Note the absent PDGF-C expression within the glomerulus. (C) Small cortical artery with smooth muscle cell PDGF-C positivity. (D) Serial section of panel C immunohistochemically stained for α-smooth muscle actin as marker of smooth muscle cells showing a virtually identical signal distributional pattern as seen for PDGF-C. (E) PDGF-C is expressed in a subset of tubular segments. (F) Serial section labeled for aquaporin-2 clearly identifies the tubular segments as collecting ducts. Magnifications: ×400 in A, E, and F; ×600 in B through D.

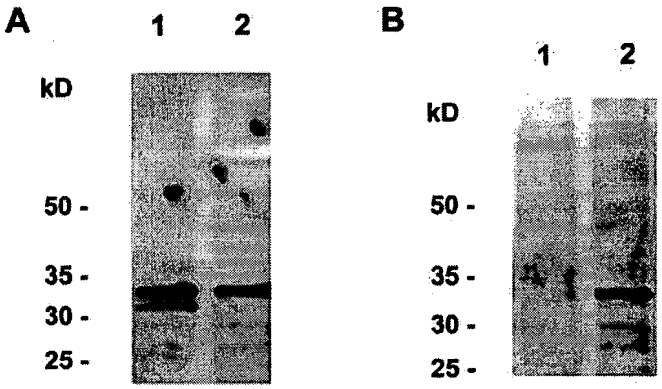

Figure 2. Western blotting for the expression of PDGF-C. (A) Cell lysates from primary rat smooth muscle cells (lane 1) and from primary rat mesangial cells (lane 2) were electrophoresed, blotted, and incubated with the anti-human PDGF-C core domain antiserum as detailed in the Materials and Methods section. A 33-kD band corresponding to the expected size of proteolytically cleaved, active PDGF-C is detected in lysates from both cell types. (B) Protein isolates from sieved glomeruli from normal rats (lane 1) and from rats at day 6 after induction of anti-Thy 1.1 glomerulonephritis induction (lane 2). Although glomeruli obtained from normal rats exhibit a very faint band only, glomerular PDGF-C expression is markedly upregulated at day 6 after glomerulonephritis induction.

strate a segmental PDGF-C staining pattern that mirrored areas of focal mesangial cell proliferates persisting after the initial injury. PDGF-C expression remained unchanged in the tubulointerstitial and the vascular compartments as compared with normal rats.

The use of the three different antisera directed against full-length human PDGF-C, core domain human PDGF-C, and mouse PDGF-C peptide resulted in virtually identical tissue localization of PDGF-C in rat renal tissues. As illustrated in Figure 3, serial sections obtained from a rat at 4 d after induction of a anti-Thy 1.1 nephritis, PDGF-C localized to activated mesangial cells was equally detected by the three different antisera preparations. This result further indicates that, due to the high degree of conservation of the amino acid sequences between mammalian species, both anti-human as well as anti-mouse PDGF-C antibody preparations are cross-reactive with rat PDGF-C.

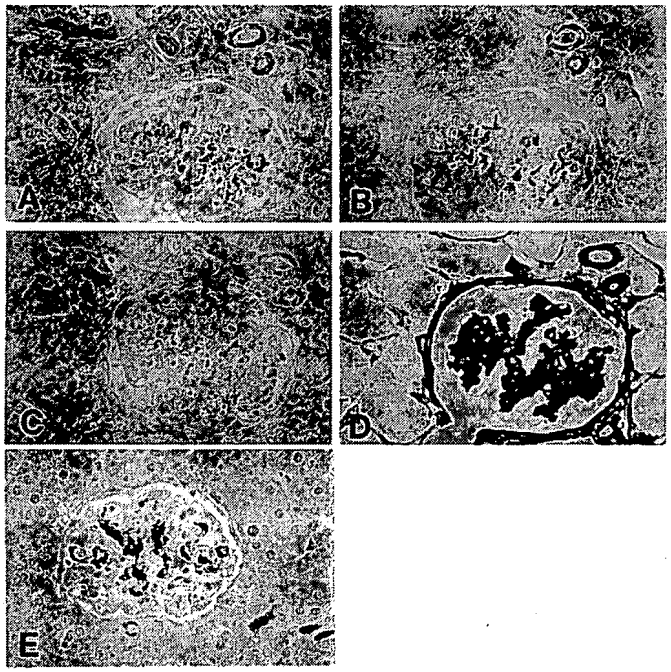

Figure 3. Mesangial PDGF-C is markedly upregulated during experimental mesangioproliferative glomerulonephritis. Serial sections of a kidney obtained from a rat 4 days after induction of a mesangioproliferative anti-Thy 1.1 glomerulonephritis. High power magnification illustrations demonstrate expression of PDGF-C within the activated glomerular mesangium. (A) Immunohistochemical analyses performed with anti-human full-length PDGF-C results in a virtually identical staining pattern compared with immunohistochemical analyses performed with anti-human core domain PDGF-C antiserum (B), or anti-mouse PDGF-C antiserum (C). The glomerular expression of PDGF-C strongly correlates with the expression of smooth-muscle α-actin, a marker of rat mesangial cell activation *in vivo* (D). Staining for α-actin identifies activated glomerular mesangial cells and arteriolar smooth muscle cells (upper right) and periglomerular myofibroblasts. A more pronounced upregulation of PDGF-C in the mesangium is present on day 6 after disease induction (E). Magnifications, × 600.

PDGF-C Acts as a Growth Factor for Mesangial Cells In Vitro

Cultured primary rat mesangial cells were growth arrested and stimulated with PDGF-AA, PDGF-BB, and PDGF-CC. Cell proliferation assays revealed that treatment with purified PDGF-CC protein led to a significant induction of mesangial cell proliferation at PDGF-CC concentrations 10 ng/ml (Figure 4A). Further increase in PDGF-CC concentrations resulted in a dose-dependent increase in mesangial cell proliferation. As expected from numerous previous experiments, both known PDGF-isoforms, PDGF-BB and to a lesser degree PDGF-AA,

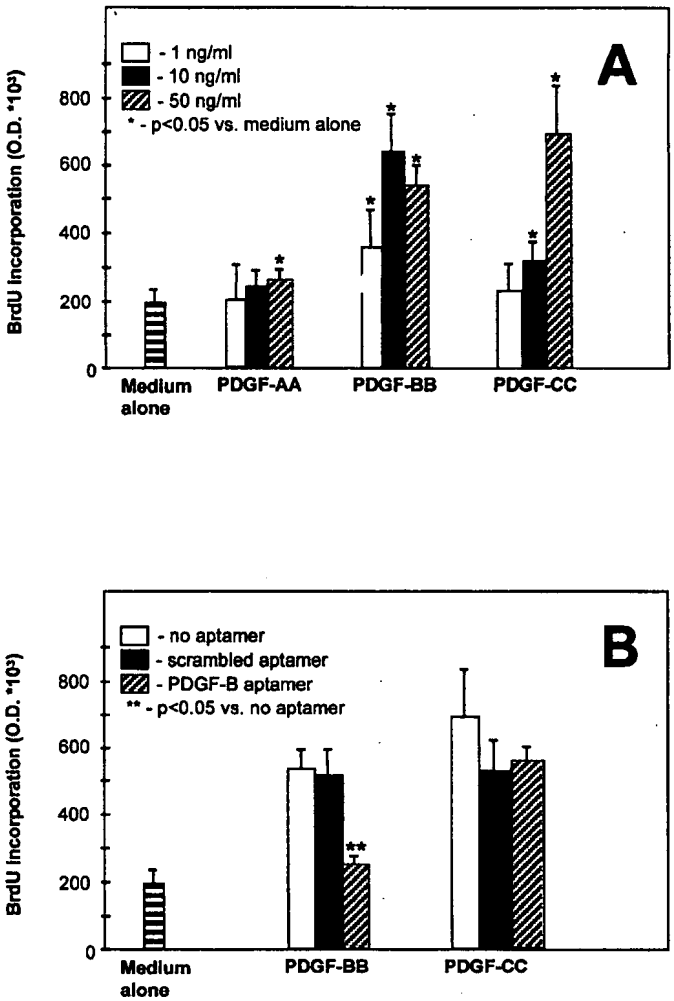

Figure 4. PDGF-C acts as growth factor for mesangial cells *in vitro*. (A) BrdU incorporation into growth arrested mesangial cells stimulated with control medium, PDGF-AA (1 to 50 ng/ml), PDGF-BB (1 to 50 ng/ml), or PDGF-CC (1 to 50 ng/ml). Data are mean ± SD of four independent experiments. $*P < 0.05$ *versus* control. (B) BrdU incorporation into growth arrested mesangial cells stimulated with control medium, PDGF-BB (50 ng/ml), PDGF-BB (50 ng/ml) with PDGF-B aptamer (1 μg/ml), PDGF-BB with control scrambled aptamer (1 μg/ml), PDGF-CC (50 ng/ml), PDGF-CC (50 ng/ml) with PDGF-B aptamer (1 μg/ml), or PDGF-CC (50 ng/ml) with control scrambled aptamer (1 μg/ml). Data are mean ± SD of four independent experiments. $**P < 0.05$ *versus* no aptamer.

were capable of inducing dose-dependent mesangial proliferation in similar concentration (Figure 4A).

PDGF-B Antagonism Does Not Alter Mesangial PDGF-C Expression

Treatment of nephritic rats with PDGF-B specific aptamer antagonists resulted in a decrease in mesangial cell proliferation and glomerular matrix accumulation at day 6 in the course of the anti-Thy 1.1 glomerulonephritis as compared with rats treated with control irrelevant aptamers. The mesangial overexpression of PDGF-C was not altered by the PDGF-B antagonism. Immunostaining scores of the PDGF-C staining demonstrated no significant differences between PDGF-B aptamer-treated nephritic rats (1.6 ± 0.4) and control aptamer-treated nephritic rats (1.6 ± 0.4). The glomerular overexpression of PDGF-B chain was significantly reduced in PDGF-B aptamer-treated nephritic rats (1.8 ± 0.9) compared with control aptamer-treated nephritic rats (2.9 ± 0.6) in parallel with a significant reduction of mesangial cell activation (α-smooth muscle-actin immunostaining scores in PDGF-B aptamer-treated nephritic rats 1.7 ± 0.2 compared with control aptamer-treated nephritis rats 2.3 ± 0.5). Additional *in vitro* experiments confirmed that the PDGF-B aptamer, although specifically antagonizing the mitogenic activity of PDGF-BB, did not affect the mitogenic effect of PDGF-CC on cultured rat mesangial cells (Figure 4B).

PDGF-C Expression in Experimental Glomerular Diseases Primarily Associated with Podocyte Injuries

We next analyzed the expression of PDGF-C in rat experimental glomerular disease models that were primarily associated with injury to visceral epithelial cells (Table 1).

Animals with toxic podocytic injury (PAN) developed massive proteinuria ranging from 210 to 300 mg/d. Glomeruli remained largely negative for PDGF-C (Figure 5A). Several glomeruli demonstrated a weak granular PDGF-C expression following a mesangial distribution. Visceral epithelial cells, *e.g.*, the primary targets of the experimental disease, remained negative for PDGF-C (Figure 5A). Both, arterial smooth muscle cell and collecting duct PDGF-C expression remained unchanged as compared with normal rats.

The development of significant proteinuria (80 to 170 mg/d) was also the functional hallmark of the immune-mediated podocyte injury (PHN). However, despite the presence of severe glomerular injury resembling human membranous nephropathy, the majority of glomeruli did not show detectable PDGF-C expression (Figure 5B). A weak granular mesangial PDGF-C expression was identified in a very limited number of glomeruli. PDGF-C expression was additionally identified in a few individual circulating leukocytes within glomerular capillary lumina. Parietal epithelial cells of some glomeruli were also positive for PDGF-C (Figure 5B). The expression of PDGF-C in collecting ducts and arterial smooth muscle cells remained normal.

Milan normotensive rats spontaneously develop focal segmental glomerulosclerosis. Visceral epithelial cells had been identified as the primary target of injury in this model (12). The majority of the glomeruli that appeared histologically normal contained no detectable PDGF-C. However, at sites of focal glomerular injury, PDGF-C was localized to visceral epithelial cells (Figure 5C). Glomerular endothelial cells and mesangial cells had no detectable PDGF-C. Another frequent finding was the PDGF-C expression of parietal epithelial cells. PDGF-C expression in vascular smooth muscle cells and in collecting ducts was unchanged.

Obese (*fa/fa*) Zucker rats develop hyperlipidemia and type IIb diabetes with progressive glomerulosclerosis and tubulointerstitial damage. The four 60-wk-old rats demonstrated significant functional alterations (increased urinary protein excretion, increased plasma creatinine) and significant histologic alterations (focal and segmental glomerulosclerosis, tubulointerstitial fibrosis, tubular atrophy) (13). The glomeruli demonstrated a wide range from histologically minimal lesions to segmental sclerosis lesions and finally globally sclerotic glomeruli. Despite the presence of severe glomerular pathology, glomerular PDGF-C expression remained negative in all cases (Figure 5D).

Increase in PDGF-C Expression at Sites of Fibrosing Tubulointerstitial Injury

Most analyzed models of glomerular disease did not develop significant tubulointerstitial pathology because of either the resolution of the injury (anti-Thy 1.1 nephritis) or the early sacrifice after disease induction (day 8 in PAN and PHN). However, 60-wk-old obese diabetic Zucker rats exhibited severe tubulointerstitial disease, including tubular atrophy, tubu-

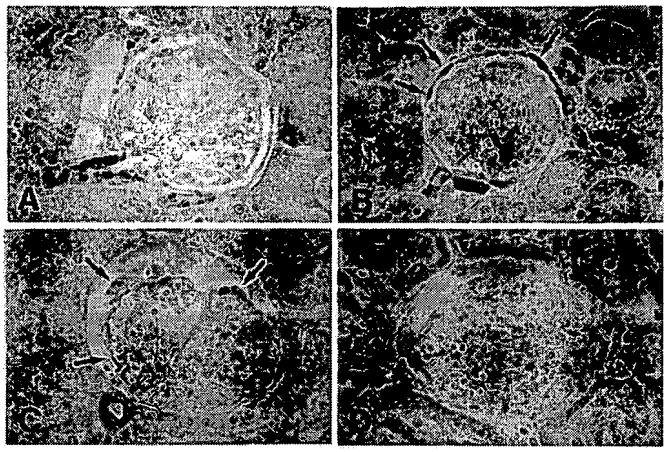

Figure 5. PDGF-C expression in experimental glomerular diseases primarily associated with podocyte injuries. (A) Representative glomerulus of a puromycin nephrosis rat immunohistochemically labeled for PDGF-C. Arteriolar smooth muscle cells express PDGF-C but glomerular cells remain negative for PDGF-C despite severe podocytic injury. (B) Absent glomerular PDGF-C expression in a glomerulus after passive Heymann nephritis disease induction. PDGF-C expression can be detected in parietal epithelial cells (arrows). (C) Representative glomerulus of a Milan rat demonstrating weak PDGF-C expression in individual visceral epithelial cells (arrows). (D) Absent PDGF-C expression in glomeruli of obese (*fa/fa*) diabetic rats. Magnification, × 600.

lar microcystic dilation, and tubulointerstitial accumulation of leukocytes and myofibroblasts. At these sites of injury, tubulointerstitial PDGF-C expression was markedly upregulated in all cases analyzed (Figure 6, A and C). PDGF-C was restricted to interstitial cells. The PDGF-C expression pattern of collecting duct epithelial cells remained unchanged compared with normal rats. We were unable to clearly identify the phenotypes of these interstitial PDGF-C expressing cells. However, serial immunohistochemical staining detected ED-1^+ macrophages in a similar distribution as PDGF-C–expressing cells (Figure 6).

Discussion

Beyond the demonstration of abundant PDGF-C transcripts in human kidney (7), no information is presently available on its intrarenal localization or pathogenetic role. In normal rat kidney, we identified arterial and arteriolar smooth muscle cells and collecting ducts as sites of constitutive PDGF-C expression. The former is consistent with a recent study by Uutela *et al.* (22) that identified PDGF-C protein by immunohistochemistry in smooth muscle cells in sections from the suprarenal artery. In addition, PDGF-C stimulated proliferation of human coronary artery and aortic smooth muscle cells *in vitro*, suggesting that PDGF-C, like the known PDGF-A and PDGF-B isoforms, can participate in vascular development and pathology (22). The detection of constitutive PDGF-C expression in collecting duct epithelial cells is novel. However, the functional role of collecting duct PDGF-C expression remains unclear, in particular, given that the animals analyzed in the present study did not show any alterations of the expression pattern in the models analyzed.

In renal disease, one of the notable findings was a marked and apparent *de novo* upregulation of PDGF-C during the proliferative response phase of anti-Thy 1.1–induced mesangioproliferative glomerulonephritis. The upregulation appeared to selectively label activated mesangial cells and as such was reminiscent of findings with PDGF-A and -B in glomerular disease (5,6). Cultured mesangial cells express both PDGF receptors, and both ligands, PDGF-A and PDGF-B, can induce proliferation of this cell type, although PDGF-B is much more potent (5,6). A central role particularly of the PDGF B-chain in mediating mesangial cell proliferation and matrix overproduction *in vivo* has been documented in several studies (5,6). Here we identify PDGF-C as the third family member that is capable of inducing mesangial cell proliferation *in vitro* with a mitogenic activity that is more potent than PDGF-AA yet lower than that of PDGF-BB. Additionally, our study identifies mesangial cells not only as a target but also as a source of PDGF-C production *in vitro* and *in vivo*, indicating the existence of an autocrine stimulatory pathway similar to that previously described for PDGF-B (5). To gain further insight into the regulation of PDGF-C *in vivo* and in particular a potential interplay with PDGF-B, we also assessed the mesangial PDGF-C expression during anti-Thy 1.1 nephritis after specific antagonism of PDGF-B. Although selective inhibition of PDGF-B had a significant effect on mesangial proliferation and matrix accumulation, the expression of mesangial PDGF-C was not altered by PDGF-B inhibition, indicating the existence of different pathways in the regulation of PDGF-B and PDGF-C.

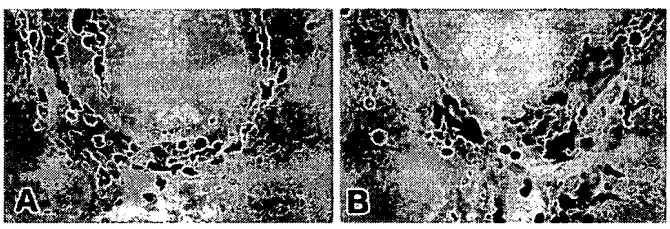

Figure 6. PDGF-C expression is increased at sites of fibrosing tubulointerstitial injury. (A) PDGF-C–expressing interstitial mononuclear cells at sites of prominent peritubular mononuclear cell infiltration in 60-wk-old obese (*fa/fa*) diabetic rats. (B) Same case as illustrated in panel A labeled for the monocyte/macrophage marker ED-1 identifies monocytes/macrophages in a similar distribution as PDGF-C expressing cells illustrated in panel *A*. Magnification, × 600.

Apart from glomerular mesangial cells, visceral and parietal epithelial cells have been identified as additional glomerular sources of apparent *de novo* PDGF-C expression in animal models with predominant injury to these cell types. These findings are again reminiscent of findings for PDGF-B, which can be expressed by visceral and parietal glomerular epithelial cells after injury (23,24). In the case of both PDGF-B and -C, the absence of functional *in vitro* and particularly *in vivo* studies presently precludes speculations on the role of PDGF-B and -C in these cell types. However, as in the case of PDGF-B (25), it is noteworthy that there was no clear correlation between the degree of glomerular sclerosis and the extent of glomerular epithelial PDGF-C expression.

A significant increase in individual PDGF-C–expressing cells was seen at sites of fibrosing interstitial injury that was most prominent in the hypercholesterolemic, diabetic rats. Although the exact cellular origin of PDGF-C expression remains to be determined, this study identified monocytes/macrophages as potential sources of PDGF-C in this scenario. However, the additional expression of PDGF-C in a small subset of fibroblasts or myofibroblasts cannot be formally excluded. Li *et al.* (7) identified PDGF-C as a potent mitogen for cultured fibroblast cells, and Gilbertson *et al.* (8) have been able to demonstrate PDGF-C–induced proliferation of several mesenchymal cell types, including human adventitial fibroblasts *in vitro*. Li *et al.* (7) have furthermore linked PDGF-C expression with fibrosis. Transgenic expression of PDGF-C in the mouse heart, using the promoter for the α-myosin heavy chain, induced strong proliferation of cardiac fibroblasts and subsequent interstitial expansion with features of interstitial fibrosis (7). In the renal interstitium, the PDGF α-receptor is constitutively expressed (7) and PDGF β-receptor is upregulated in progressive renal failure (19). Therefore, the *de novo* occurrence of PDGF-C–producing cells in damaged renal tubulointerstitium may contribute to the pathogenesis of renal tubulointerstitial fibrosis.

In conclusion, this study links pathologic overexpression of the new growth factor PDGF-C to the induction of mesangial cell proliferation *in vivo* and *in vitro* as well as to tubulointer-

stitial inflammation and fibrosis. Compounds that specifically inhibit PDGF-C *in vivo* will help to further define the role of PDGF-C in the concert of actions of the members of the PDGF family of cytokines.

Acknowledgments

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PDGF-D is a specific, protease-activated ligand for the PDGF β-receptor

Erika Bergsten*†, Marko Uutela†‡, Xuri Li*, Kristian Pietras§, Arne Östman§, Carl-Henrik Heldin§, Kari Alitalo‡ and Ulf Eriksson*¶

*Ludwig Institute for Cancer Research, Stockholm Branch, PO Box 240, S-171 77 Stockholm, Sweden
‡Molecular/Cancer Biology Laboratory, Haartman Institute, University of Helsinki, PO Box 21 (Haartmaninkatu 3), SF-00014 Helsinki, Finland
§Ludwig Institute for Cancer Research, Uppsala Branch, PO Box 595, S-751 24 Uppsala, Sweden
†These authors contributed equally to this work
¶e-mail: ueri@licr.ki.se

The term 'platelet-derived growth factor' (PDGF) refers to a family of disulphide-bonded dimeric isoforms that are important for growth, survival and function in several types of connective tissue cell. So far, three different PDGF chains have been identified — the classical PDGF-A and PDGF-B[1,2] and the recently identified PDGF-C[3]. PDGF isoforms (PDGF-AA, AB, BB and CC) exert their cellular effects by differential binding to two receptor tyrosine kinases. The PDGF α-receptor (PDGFR-α) binds to all three PDGF chains, whereas the β-receptor (PDGFR-β) binds only to PDGF-B[1]. Gene-targeting studies using mice have shown that the genes for PDGF-A and PDGF-B, as well as the two PDGFR genes, are essential for normal development[4]. Furthermore, overexpression of PDGFs is linked to different pathological conditions, including malignancies, atherosclerosis and fibroproliferative diseases[1]. Here we have identify and characterize a fourth member of the PDGF family, PDGF-D. PDGF-D has a two-domain structure similar to PDGF-C[3] and is secreted as a disulphide-linked homodimer, PDGF-DD. Upon limited proteolysis, PDGF-DD is activated and becomes a specific agonistic ligand for PDGFR-β. PDGF-DD is the first known PDGFR-β-specific ligand, and its unique receptor specificity indicates that it may be important for development and pathophysiology in several organs.

We identified PDGF-D as a human expressed sequence tag (EST) in a BLAST search of the National Center for Biotechnology Information EST databases (accession no. AI488780). This EST encodes a polypeptide with ~50% identity to the carboxy-terminal portion of PDGF-C. Using a DNA probe derived from the EST sequence, we isolated several partial complementary DNA clones, and generated the extreme 5′ portion of the cDNA by rapid amplification of cDNA ends (RACE). The full-length cDNA for human PDGF-D encoded a polypeptide of 370 amino acids, which like PDGF-C has a two-domain structure with an amino-terminal CUB (InterPro IPR000859) domain (residues 56–167; reviewed in ref. 5) and a C-terminal PDGF/vascular endothelial growth factor (VEGF)-homology domain (residues 272–362, also known as the core domain). The identity shared by the full-length amino-acid sequences of PDGF-C and PDGF-D is ~43% (Fig. 1a). Similarity is highest in the distinct protein domains, whereas the N-terminal region, including the hydrophobic signal sequence, and the hinge region between the two domains have less identity. We identified a putative site for signal peptidase cleavage between residues 22 and 23; cleavage at this site would result in a secreted protein of 348 residues with a calculated relative molecular mass (M_r) of 40,270. We also identified a single putative site for *N*-linked glycosylation in the core domain of PDGF-D (residues 276–278).

The PDGF/VEGF domain of PDGF-D is ~50% identical to the corresponding domain in PDGF-C, and 20–23% identical to the core domains of the classical PDGFs and VEGFs (Fig. 1b). Only seven out of the eight invariant cysteine residues found in other PDGF/VEGF domains are present in PDGF-D (the fifth conserved cysteine residue is replaced by glycine). Similar to the unique insertion of three residues in PDGF-C, PDGF-D has an insertion of three amino acids (sequence NCG) between conserved cysteine residues three and four. In total, there are ten cysteine residues in the core domain, including the extreme C-terminal region, indicating a unique arrangement of the cysteines in the disulphide-bonded PDGF-D dimer. Phylogenetic analysis of the core domains of PDGFs and the VEGFs showed that the PDGF-D core domain forms a subgroup of the PDGFs together with PDGF-C (Fig. 1c).

We expressed the full-length form of PDGF-D, containing a C-terminal six-histidine (His_6) tag, in baculovirus-infected Sf9 insect cells. We purified the expressed protein on Ni–NTA–agarose columns and subjected it to SDS–polyacrylamide-gel electrophoresis (SDS–PAGE) under both reducing and non-reducing conditions (Fig. 2a). Non-reduced PDGF-D migrated with an M_r of 90,000 (90K), whereas the reduced protein migrated as a species of M_r 55K. We also expressed and purified a His-tagged version of full-length PDGF-D containing specific cleavage sites for the factor Xa protease in the hinge region that precedes the core domain (hereafter called fXPDGF-D, cleveage site IEGR × 2, replacing amino acids 251–258). The fXPDGF-D mutant showed similar electrophoretic properties to wild-type PDGF-D under reducing and non-reducing conditions (data not shown). Thus PDGF-D, like PDGF-A, B and C, forms disulphide-linked homodimers (PDGF-DD), and the introduced factor Xa cleavage sites did not affect the ability of fXPDGF-D chains to form such dimers.

It is known that full-length PDGF-CC, which has a domain structure similar to that of PDGF-DD, requires limited proteolysis to release the core domains of the protein, which then interact with its receptor[3]. Attempts to express independently the core domain of PDGF-D in baculovirus-infected insect cells were unsuccessful, as the truncated protein was retained intracellularly and was not secreted (E.B. and U.E., unpublished observations). We generated protease-treated preparations of wild-type PDGF-DD and fXPDGF-DD, analysed them by SDS–PAGE under reducing conditions, and immunoblotted them using an antipeptide antiserum raised against a sequence of PDGF-D just N-terminal to the first cysteine residue in the core domain. Plasmin digestion of wild-type PDGF-DD (data not shown) and factor Xa-digestion of fXPDGF-DD (Fig. 2b) generated distinct species with M_r values of 15K and 21K, respectively.

To investigate whether full-length PDGF-D is proteolytically processed *in vivo*, we overexpressed PDGF-D tagged with a Myc epitope in the hearts of transgenic mice using the α-myosin heavy chain (αMHC) promoter[6]. We analysed heart tissue extracts from

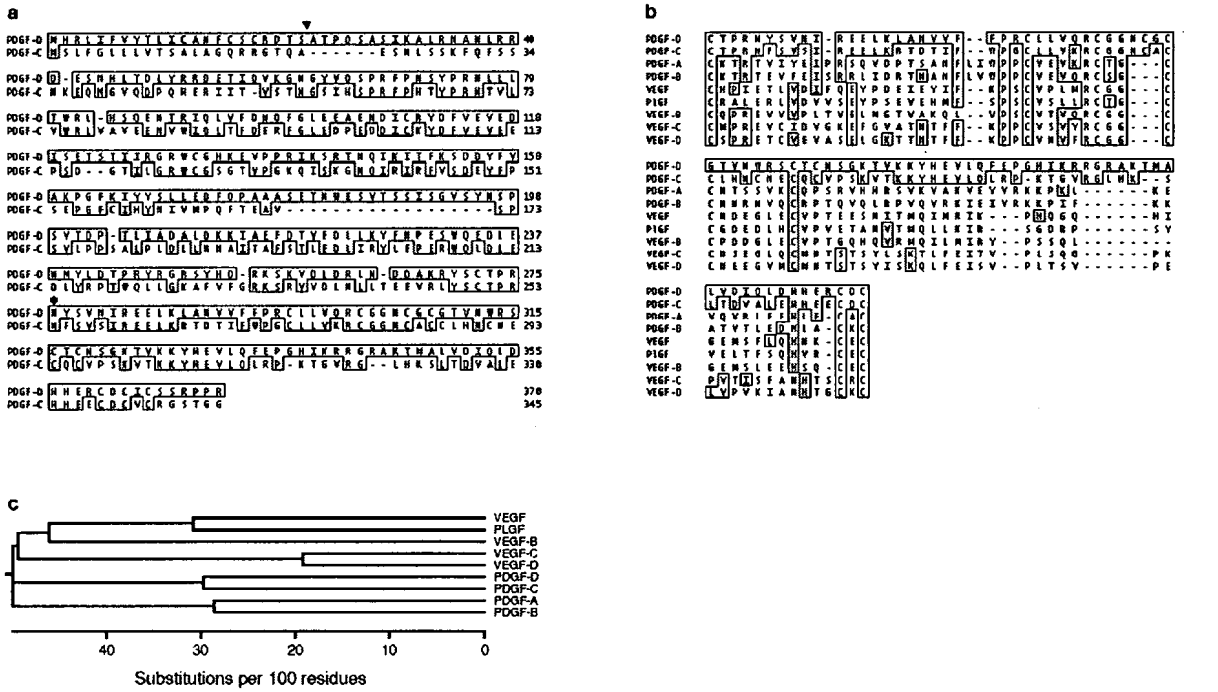

Figure 1 **Amino-acid sequence of human PDGF-D. a**, Alignment of the amino-acid sequences of human PDGFD and PDGF-C. Identical residues are boxed. The putative signal peptidase cleavage site between residues 22 and 23 (triangle) and the putative *N*-linked glycosylation site at residue 276 are marked (star). **b**, Alignment of the amino-acid sequences of members of the PDGF/VEGF family. Only regions that encompass the conserved cysteine-rich domain, which is involved in inter- and intra-disulphide bonds, are shown. Identical residues to those in PDGF-D are boxed, including the invariant cysteine residues found in all members of the PDGF/VEGF family. A unique feature of PDGF-D is that the fifth invariant cysteine residue is replaced by glycine. Similar to PDGF-C, PDGF-D has an insertion of three residues between cysteines three and four of the conserved cysteine motif. **c**, Phylogenetic analysis of growth-factor-homology domains in the PDGF/VEGF family. PDGF-D and PDGF-C form a subgroup of the PDGFs with an overall amino-acid sequence identity of ~43%.

transgenic and normal animals by immunoblotting using a monoclonal anti-Myc antibody to detect transgenic PDGF-D. Myc-tagged full-length PDGF-D (M_r 62K) was abundantly expressed in the transgenic hearts, as were several processed species, including two prominent fragments of M_r 36K and 30K (Fig. 2c). These results indicate that the myocardium of the transgenic mice may express enzymes that are capable of processing latent full-length PDGF-DD *in vivo*. A detailed phenotypic description of the transgenic animals will be published elsewhere.

We investigated the receptor specificity of full-length and plasmin-digested wild-type PDGF-DD and of full-length and factor Xa-cleaved fXPDGF-DD as competitors in PDGF receptor-binding assays. Using increasing concentrations of the two PDGF-DD preparations, we observed that full-length wild-type PDGF-DD and fXPDGF-DD failed to compete for binding of ^{125}I-labelled PDGF-BB to PDGFR-α and to PDGFR-β. In contrast, plasmin-digested PDGF-DD and factor Xa-digested fXPDGF-DD specifically competed for binding of ^{125}I-labelled PDGF-BB to PDGFR-β, but failed to compete for binding to PDGFR-α (Fig.2 d, e). Relative to PDGF-BB, protease-activated PDGF-DD was 10–12-fold less efficient as a competitor. As the core domain of PDGF-DD accounts for less than one-third of the full-length protein, these results indicate that activated PDGF-DD may be roughly threefold less efficient as a competitor in the ligand-binding assay, compared with PDGF-BB. Control experiments showed that plasmin and factor Xa present in the protease-digested PDGF-DD fractions did not affect the binding of ^{125}I-labelled PDGF-BB to PDGFR-β-expressing cells (data not shown). We also analysed the ability of the core domain of PDGF-DD to bind to VEGF receptors. No significant interaction was observed between the PDGF-DD core domain with VEGF receptors 1, 2 or 3 (M.U. and K. A., unpublished observations).

We next investigated the ability of full-length and protease-digested PDGF-DD to induce tyrosine phosphorylation of PDGFR-β. After stimulation of porcine aortic endothelial (PAE) cells expressing PDGFR-β with different preparations of PDGF-DD, we immunoprecipitated PDGFR-β from cell lysates, and subjected the samples to SDS–PAGE and immunoblotting with monoclonal antibodies against phosphotyrosine. Both plasmin-digested PDGF-DD and factor Xa-digested fXPDGF-DD stimulated tyrosine phosphorylation of receptors in a dose-dependent manner, as did PDGF-BB (Fig. 2f), whereas the full-length proteins failed to do so (Fig. 2f and data not shown). A similar analysis of receptor activation in human foreskin fibroblasts revealed strong tyrosine phosphorylation of the PDGFR-β, whereas PDGFR-α was only marginally phosphorylated (Fig. 2g). As a control, we stimulated heterodimeric receptor complexes with PDGF-AB heterodimers; both PDGF receptors were activated under these conditions. We conclude that PDGF-DD is a PDGFR-β-specific agonist and that proteolytic processing, releasing the core domains of PDGF-DD from the N-terminal CUB domains, is necessary for unmasking the receptor-binding epitopes of the core domain, as is the case for PDGF-CC[3].

We used a ^{32}P-labelled DNA probe to study the expression of PDGF-D transcripts in several human tissues by northern blotting (Fig. 3a). Highest expression of a principal 4.0-kilobase (kb) transcript occurred in heart, pancreas and ovary, whereas lower

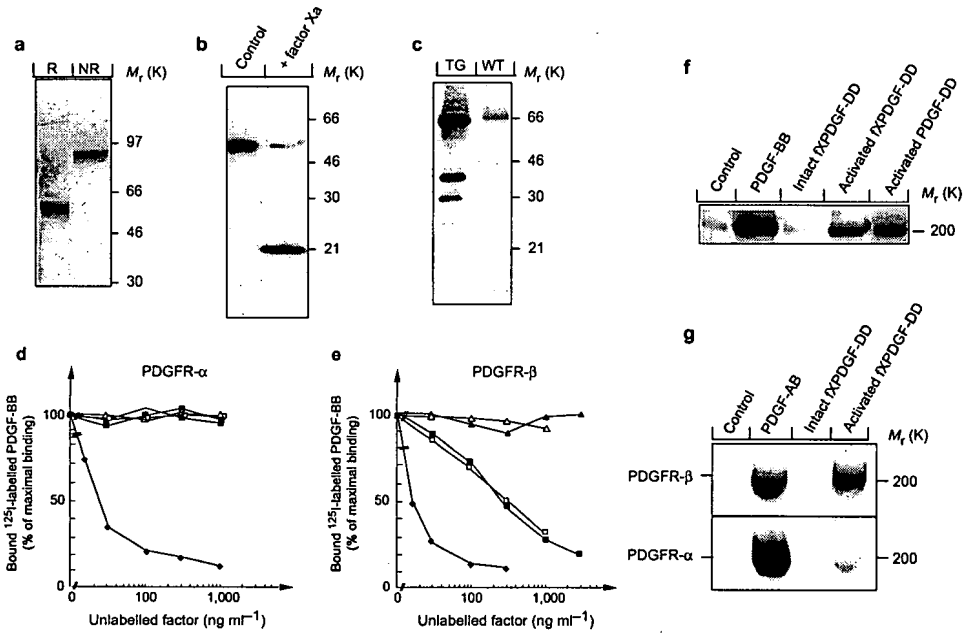

Figure 2 **Recombinant expression, *in vitro* and *in vivo* processing, and receptor analysis of PDGF-DD. a**, SDS–PAGE analysis of human recombinant PDGF-DD under reducing (R) and non-reducing (NR) conditions. PDGF-D was visualized by staining with Comassie brilliant blue. **b**, SDS–PAGE under reducing conditions and immunoblotting of human recombinant PDGF-DD containing introduced sites for factor Xa cleavage. Cleavage with factor Xa generates a species of M_r 21K, whereas the full-length protein has an M_r of 55K. The recombinant protein was detected using an antipeptide antiserum raised against a sequence N-terminal to the first cysteine residue in the core domain of PDGF-D. **c**, Mouse-heart tissue extracts were analysed by SDS–PAGE under reducing conditions and then immunoblotted. Transgenic PDGF-D (TG) was tagged at its C terminus with a Myc epitope and was detected using a specific anti-Myc antibody. A faint background band (M_r 66K) was observed in tissue extracts from wild-type heart (WT). The processed forms of PDGF-D have M_r values of 30K and 36K species. **d**, **e**, Competitive inhibition of binding of ^{125}I-labelled PDGF-BB to PAE cells expressing PDGFR-α (**d**) or PDGFR-β (**e**) by increasing concentrations of PDGF-BB (diamonds), full-length PDGF-DD (filled triangles), plasmin-treated PDGF-DD (filled squares), full-length fXPDGF-DD (open triangles) or factor Xa-treated fXPDGF-DD (open squares). **f**, Induction of tyrosine phosphorylation of PDGFR-β in PAE cells by full-length fXPDGF-DD, factor Xa-activated fXPDGF-DD or plasmin-activated PDGF-DD (300 ng ml^{-1}). Unstimulated PAE cells (control) and cells stimulated with 10 ng ml^{-1} PDGF-BB were used as controls. **g**, Induction of tyrosine phosphorylation of PDGF receptors in AG1519 human-foreskin fibroblasts stimulated with full-length fXPDGF-DD (300 ng ml^{-1}) or factor Xa-activated fXPDGF-DD (300 ng ml^{-1}). Unstimulated cells (control) or cells stimulated with PDGF-AB heterodimers (100 ng ml^{-1}) were used as controls. In **f** and **g**, PDGF receptors were immunoprecipitated from detergent-lysed cells, subjected to SDS–PAGE, and immunoblotted with monoclonal PY99 antibodies against phosphotyrosine.

expression levels were observed in several other tissues, including placenta, liver, kidney, prostate, testis, small intestine, spleen and colon. No expression was detected in brain, lung or skeletal muscle. In comparison, the 3.5-kb PDGF-B transcript was abundantly expressed in heart and placenta, whereas lower levels were observed in all other tissues. PDGF-D and PDGF-B were prominently co-expressed in heart, pancreas and ovary.

We analysed the tissue expression of PDGF-D in mouse embryos during mid-gestation (embryonic day 14.5) using immunohistochemistry with affinity-purified rabbit antibodies against full-length human PDGF-DD. These antibodies did not recognize PDGF-C in immunoblot analyses (Fig. 3b). Intense staining for PDGF-D was observed in several tissues, including the developing heart, lung, kidney and some muscle derivatives. Here we focus on the developing kidney; a broader expression analysis will be presented elsewhere. We observed intense staining of the highly vascularized fibrous capsule that surrounds the embryonic kidney, of the adjacent adrenal gland, and of the most peripheral aspect of the metanephric mesenchyme of the cortex (Fig. 3c, d). Staining was also present in cells located in the basal aspect of the branching ureter (Fig. 3e), whereas the developing nephron, including the ureter buds, glomeruli and Henle's loops, were devoid of staining. Previous analyses have shown that PDGFR-β is expressed by the metanephric mesenchyme and the developing vascular smooth-muscle cells and mesangial cells of the developing renal cortex[7,8]. In contrast, renal expression of PDGF-B is restricted to endothelial cells[7]. The non-overlapping patterns of expression of the two PDGFR-β ligands indicates that PGDF-B and PGDF-D may provide distinct signals to PDGFR-β-expressing perivascular cells. On the basis of their differential localization, it is appealing to speculate that PDGF-D may have a paracrine function in proliferation and/or commitment of PDGFR-β-expressing perivascular progenitor cells of the undifferentiated metanephric mesenchyme. As indicated by the phenotype of PDGF-B-deficient mice, PDGF-B may then provide proliferative signals and spatial cues for the branching vascular tree of the kidney, thus allowing proliferation and co-recruitment of PDGFR-β-expressing perivascular cells to form the mesangium of the glomeruli, and the smooth-muscle cells of the efferent and afferent arterioles[7,9].

The expression of PDGF-D partially overlaps with that of PDGF-C in the cortical area of the developing kidney[3]. The different receptor specificities of PDGF-C and PDGF-D, and their apparent inability to form heterodimers (X. L. and U. E., unpublished observations) indicate that these two recently identified PDGFs may provide distinct signals for migration and proliferation for at least two different cell populations in the undifferentiated metanephric mesenchyme — interstitial cell progenitors expressing PDGFR-α, and perivascular progenitor cells expressing PDGFR-β.

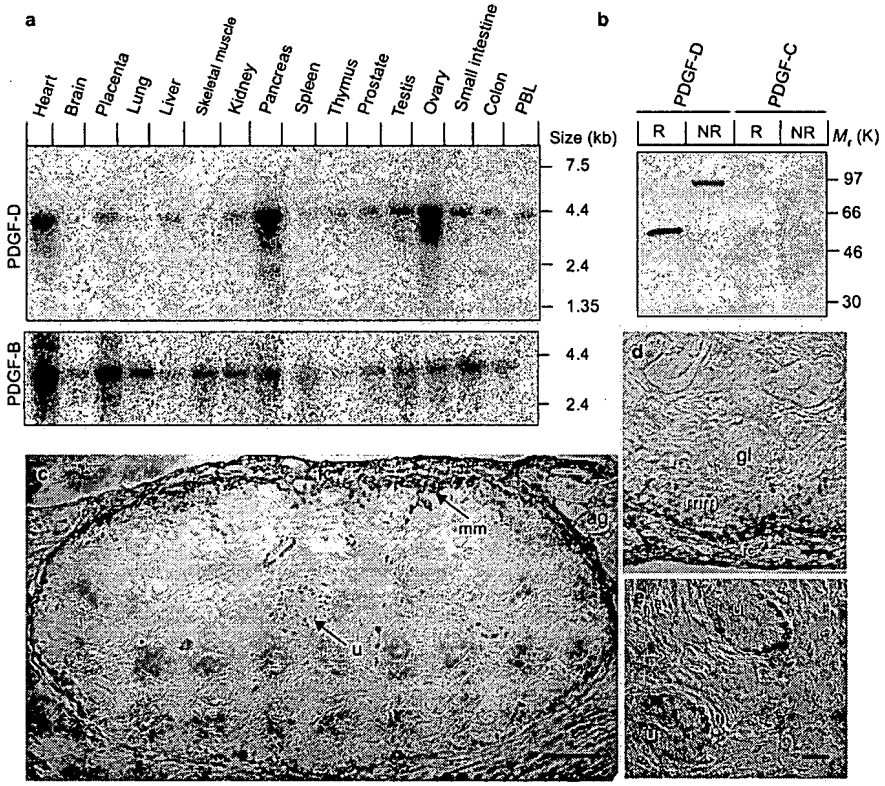

Figure 3 **Expression of PDGF-D in adult and embryonic tissues. a**, Northern-blot analysis of PDGF-D and PDGF-B transcripts in several adult human tissues. The blot was sequentially hybridized with ^{32}P-labelled probes for PDGF-D (upper panel) and PDGF-B (lower panel). PDGF-D transcripts are most highly expressed in heart, pancreas and ovary, whereas PDGF-B transcripts are most abundant in heart, placenta and lung. Co-expression of PDGF-D and PDGF-B was observed in several tissues, most notably heart, pancreas and ovary. PBL, peripheral blood leukocytes. **b**, Immunoblot analysis of purified PDGF-DD and PDGF-CC proteins using affinity-purified rabbit antibodies against PDGF-D. The analysis was carried out using reduced (R) and non-reduced (NR) proteins. Anti-PDGF-D antibodies do not cross-react with PDGF-C. **c–e**, Immunohistochemical localization of PDGF-D in the developing mouse kidney at embryonic day 14.5. PDGF-D (red-brown) is most abundantly found in the fibrous capsule (fc) surrounding the developing kidney and the adjacent adrenal gland (ag). Staining is also present in the metanephric mesenchyme (mm) in the cortical region of the kidney and in the branching ureter (u) in the medullar region. Notably, no PDGF-D staining was observed in the developing nephron, including the ureter buds, glomeruli (gl) and Henle's loops. Scale bars represent 100 μm (**c**) and 50 μm (**d**, **e**).

The phenotypic differences between the kidneys of mice lacking PDGFR-α and those lacking PDGF-A support the idea that another PDGFR-α ligand, such as PDGF-C, has a unique function in the formation of the renal mesenchyme[3]. Comparison of mice lacking PDGFR-β and those lacking PDGF-B has not revealed a similar phenotypic discrepancy, indicating that PDGF-D and PDGF-B may have at least partially redundant functions during the early stages of kidney development[9,10]. Targeted deletion of PDGF-D will be needed to test this hypothesis.

Our discovery of PDGF-D as a new PDGFR-β agonist provides further, and rather unexpected, insight into the complexity of the PDGF/PDGFR system during organogenesis, for instance of the kidney. PDGFR-α and PDGFR-β have different signalling capacities, for example, regarding chemotaxis of fibroblasts and smooth-muscle cells, which is stimulated by PDGFR-β and inhibited by PDGFR-α[1]. It is therefore possible that PDGF-D, which activates PDGFR-β but unlike other PDGF isoforms does not efficiently activate PDGFR-α, has important functions, for example in blood-vessel development and pathophysiology.

Note added in proof. The identification and partial characterization of PDGF-D are also described on page 517 of this issue by LaRochelle et al.[11]. □

Methods

Cloning of human PDGF-D cDNA.

Two primers, 5′-GTCGTGGAACTGCAACTGG (forward) and 5′-CTCAGCAACCACTTGTGTTC (reverse), derived from the identified human EST sequence (accession no. AI488780), were used in polymerase chain reactions (PCR) to amplify a 327-base pair (bp) fragment using DNA from a human fetal lung 5′-STRETCH PLUS λgt10 cDNA library (Clontech) as a template. The fragment was cloned into the pCR 2.1 vector (TA Cloning Kit, Invitrogen). The PCR fragment was labelled to high specific activity by random priming (Amersham) and the same cDNA library was screened by plaque hybridization to isolate several partial cDNA clones. The longest subcloned insert was 1,934 bp in length and encoded the C-terminal 322 amino acids of PDGF-D, whereas the 5′ part was missing. The 5′ part of the cDNA was then amplified by RACE using human-heart cDNA as a template (Marathon-Ready cDNA, Clontech) with adaptor primer 5′-CCATCCTAATACGACTCACTATAGGGC (forward) and a second primer derived from the partial cDNA clones, 5′-AGTGGGATCCGTTACTGATGGA-GAGTTAT (reverse). Amplification reactions were carried out using Advantage-GC cDNA PCR kit, (Clontech) and a 790-bp amplified fragment was cloned into the TOPO TA vector (TOPO TA Cloning Kit, Invitrogen). A full-length cDNA for PDGF-D was constructed by fusing the two partial cDNA clones at a common restriction site. Nucleotide sequences were determined using internal and vector-specific primers.

Expression of human PDGF-D in baculovirus-infected insect cells.

The part of the cDNA encoding amino acids 24–370 was amplified by PCR using *Taq* DNA polymerase (Amersham Pharmacia Biotech). The primers used were as follows: 5′-GATATCTAGAAG-CAACCCCGCAGAGC (forward, including an *Xba*I site for in-frame cloning, underlined), and 5′-GCTCGAATTCTAAATGGTGATGGTGATGATGTCGAGGTGGTCTTGA (reverse, including an *Eco*RI site, underlined, and sequences encoding a C-terminal His_6 tag). The amplified products were cloned in the pCR 2.1 vector (Invitrogen) and the *Xba*I–*Not*I-digested fragment was cloned into the baculovirus

expression vector pAcGP67A (Pharmigen). For generation of mutant PDGF-D containing sites for factor Xa cleavage, the pCR 2.1 vector with the PDGF-D insert was modified at the position corresponding to amino acids 251–258 (sequence YHDRKSKV) into two tandem factor Xa cleavage sites (sequence IEGR × 2) by single-strand mutagenesis[12,11] using the primer 5′-CCTATCCAGGTCACGTCCTTCGATCCCGCCTTCGATTGACCTGCCTCG. After restriction digestion, the modified insert was subcloned into the baculovirus expression vector. Procedures used for generation of recombinant baculovirus expressing PDGF-D and for production, purification and analysis of recombinant PDGF-DD and PDGF-CC were as described[3].

Rabbit antisera against full-length PDGF-DD and against a synthetic peptide derived from the PDGF-D sequence (amino acids 254–272, sequence CRKSKVDLDRLNDDAKRYSC) were generated as described[3]. Antibodies against full-length PDGF-DD were affinity-purified on a column of purified full-length PDGF-DD coupled to CNBr-activated sepharose 4B (Pharmacia).

Receptor binding and receptor activation of PDGF-DD.

Ligand-binding competition experiments were carried out essentially as described[3,14], using PAE cells expressing human PDGFR-α or PDGFR-β. Aliquots of full-length or plasmin-digested PDGF-DD or of full-length or factor Xa-digested fXPDGF-DD (see below) were diluted in binding buffer and assayed for inhibition of binding of ^{125}I-labelled PDGF-BB to cells expressing PDGFR-α or PDGFR-β.

Growth-factor-induced tyrosine phosphorylation of PDGFR-β was analysed in PAE cells, essentially as described for PDGF-CC-induced stimulation of PDGFR-α[3]. Activation of PDGF receptors in fibroblasts was analysed using AG1519 human-foreskin fibroblasts (Coriell Cell Repository, Camden, New Jersey). Cells were stimulated with PDGF-AB heterodimers (100 ng ml^{-1}), full-length fXPDGF-DD (300 ng ml^{-1}) or factor Xa-activated fXPDGF-DD (300 ng ml^{-1}). Induction of PDGFR-α and PDGFR-β tyrosine phosphorylation was subsequently analysed as described above using specific antisera against the different PDGF receptors[15].

Full-length PDGF-DD was digested with plasmin as described[3]. The mutated version fXPDGF-DD, containing sites for factor Xa cleavage, was digested with factor Xa according to the manufacturer's instructions (Roche). The progression of the digestions was analysed by SDS–PAGE under reducing conditions, followed by immunoblotting using the antipeptide antiserum (see above). Receptor-binding and receptor-activation experiments were repeated at least three times with similar results.

Transgenic expression of full-length PDGF-D in mouse heart.

The sequence encoding the human Myc epitope was introduced at the 3′ end of the coding region of human PDGF-D cDNA by PCR mutagenesis using the following primers: 5′-GATAGTCGACTCCCAAATGCACCGG (forward) and 5′-AGTTCTGGTGGAGCTCTTGTTTTCGAATAAAGACTTCTTCTGAACATTCAGCTGATGC (reverse). Procedures used to generate the transgenic consruct driven by the α-MHC promoter[6] and to generate and analyse transgenic mice were essentially as described[3].

Northern blotting and immunohistochemistry.

A human multiple-tissue northern blot (MTN, Clontech) was sequentially hybridized, using the ^{32}P-labeled 327-bp PCR fragment of PDGF-D cDNA and full-length PDGF-B cDNA as probes at high stringency and ExpressHyb hybridization solution, according to the manufacturer's instructions (Clontech).

For immunohistochemistry, affinity-purified rabbit antibodies against human PDGF-DD (3–9 μg ml^{-1}), prepared as described above, were applied to tissue sections prepared from paraformaldehyde-fixed and paraffin-embedded mouse embryos. Staining was carried out as described[16]. In control experiments antibodies were pre-incubated with a 30-fold molar excess of full-length PDGF-DD. This blocked the staining, whereas similar pre-incubation with full-length PDGF-CC did not. Photomicrographs were obtained using a Zeiss microscope equipped with differential interference contrast optics.

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Correspondence and requests for materials should be addressed to U.E. The nucleotide and amino-acid sequences of human PDGF-D have been deposited at GenBank under accession no AF336376.

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Specific Antagonism of PDGF Prevents Renal Scarring in Experimental Glomerulonephritis

TAMMO OSTENDORF,* UTA KUNTER,* HERMANN JOSEPH GRÖNE,† FERDINAND BAHLMANN,‡ HIROSHI KAWACHI,§ FUJIO SHIMIZU,§ KARL MARTIN KOCH,‡ NEBOJŠA JANJIC,‖ and JÜRGEN FLOEGE*

**Medizinische Klinik II, University of Aachen, Aachen, †Department of Pathology, Deutsches Krebsforschungszentrum, Heidelberg, and ‡Division of Nephrology, Medizinische Hochschule, Hannover, Germany; §Department of Cell Biology, Institute of Nephrology, Niigata School of Medicine, Niigata, Japan; and ‖NeXstar Pharmaceuticals, Boulder, Colorado.*

Abstract. Glomerular mesangial cell proliferation and/or mesangial matrix accumulation characterizes many progressive renal diseases. Rats with progressive mesangioproliferative glomerulonephritis were treated from day 3 to day 7 after disease induction with a high-affinity oligonucleotide aptamer-antagonist against platelet-derived growth factor-B chain (PDGF-B). In comparison with nephritic rats that received vehicle or a scrambled aptamer, treatment with the PDGF-B aptamer led to a significant reduction of mesangioproliferative changes, glomerular hypertrophy, podocyte damage, and glomerular macrophage influx on day 8. Both nephritic control groups subsequently developed progressive proteinuria and decreased renal function. On day 100, glomerulosclerosis, tubulointerstitial damage, glomerular and interstitial accumulation of types III and IV collagen, and overexpression of transforming growth factor-β were widespread. All of these chronic changes were prevented in rats that received the PDGF-B aptamer, and their functional and morphologic parameters on day 100 were largely indistinguishable from non-nephritic rats. These data provide the first evidence for a causal role of PDGF in the pathogenesis of renal scarring and point to a new, highly effective therapeutic approach to progressive, in particular mesangioproliferative, renal disease.

Populations of patients with end-stage renal disease continue to grow in most Western countries (1). The most frequent causes of renal failure are diabetic nephropathy and glomerulonephritides (1). Both diabetic nephropathy and the majority of progressive glomerulonephritides, such as IgA nephropathy, membranoproliferative glomerulonephritis, variants of idiopathic focal sclerosis, and lupus nephritis, are histologically characterized by glomerular mesangial cell proliferation and/or matrix accumulation (2,3). Of the various factors that affect mesangial cell behavior, platelet-derived growth factor-B chain (PDGF-B) seems to have a particularly important role (reviewed in references 4 and 5). First, mesangial cells produce PDGF, and various growth factors induce mesangial proliferation via induction of PDGF-B chain synthesis. Second, PDGF-B chain and its receptor are overexpressed in many glomerular diseases. Third, infusion of PDGF-BB or glomerular transfection with a PDGF-B chain cDNA induces selective mesangial cell proliferation and matrix accumulation *in vivo*. Fourth, PDGF-B chain or β-receptor knockout mice fail to develop a mesangium. Finally, antagonism of PDGF-B chain with neutralizing antibodies can reduce mesangial cell proliferation and matrix accumulation in a (reversible, nonprogressive) rat model of mesangioproliferative nephritis, the anti–Thy-1.1 model.

Based on the above observations, specific antagonism of PDGF-B chain might represent a novel therapeutic approach to progressive renal diseases characterized by mesangial expansion. However, despite the considerable information on PDGF in glomerular disease, all intervention studies so far have been confined to short-term observations and acute effects. Consequently, it is unclear whether antagonism of PDGF in glomerular disease indeed exerts long-term beneficial effects and, more important, whether it is safe, given that mesangioproliferative changes frequently represent a response to injury (6) and as such may be analogous to a healing reaction.

We recently described a nuclease-resistant, high-affinity oligonucleotide aptamer that specifically inhibits PDGF activity (7,8). This aptamer potently suppressed mesangial cell proliferation and matrix accumulation in the anti–Thy-1.1 nephritis model (8). Furthermore, in comparison with our previous study with neutralizing anti-PDGF antibodies in anti–Thy-1.1 nephritis (9), the aptamer antagonist offered the advantage of nonimmunogenicity (Drolet D, unpublished observations), which permits long-term observations. We investigated an anti–Thy-1.1 nephritis model, which, when left untreated, will progress to renal failure (10). Using this model, we addressed the question of whether transient antagonism of PDGF using

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Correspondence to Dr. Jürgen Floege, Medizinische Klinik II, Klinikum der RWTH, Pauwelsstrasse 30, D-52074, Aachen, Germany. Phone: 49-24-1808-9530; Fax: 49-24-1888-8446; E-mail: juergen.floege@post.rwth-aachen.de
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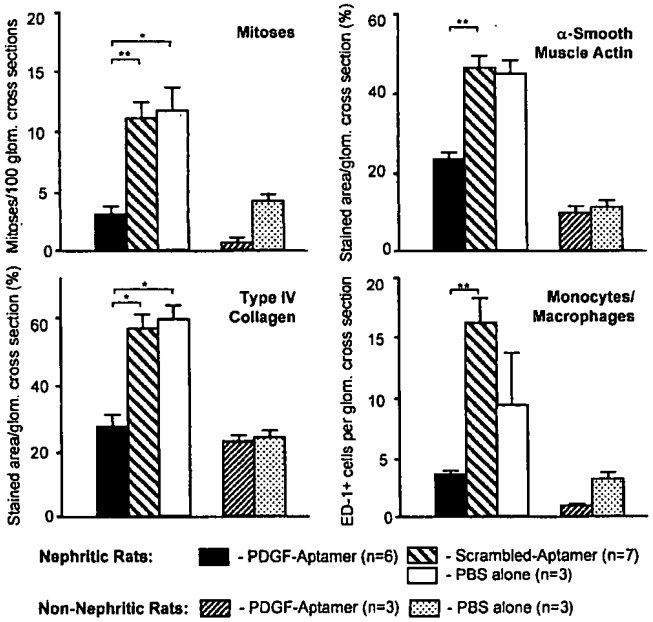

Figure 1. Glomerular immunohistologic changes on day 8 after disease induction in rats that received the PDGF aptamer, the scrambled aptamer, or phosphate-buffered saline (PBS) alone. *, $P < 0.05$; **, $P < 0.01$.

the specific aptamer antagonist affects the long-term evolution of progressive renal disease.

Materials and Methods

Aptamer-Based Antagonist against PDGF

The synthesis and characterization of the PDGF-B aptamer (NX1975) have been described in detail (7,8). Modifications of the original aptamer (7) involved substitutions of unmodified nucleotides with 2-fluoropyrimidines and 2'-O-methylpurines to improve nuclease resistance as well as coupling of the molecule to 40 kD polyethylene glycol (PEG) to prolong the *in vivo* half-time of the aptamer (8). The PDGF-B aptamer bound to rat and human recombinant PDGF-BB with the same affinity (K_d approximately 0.1 nM) (8). Based on photo cross-linking experiments (7), the aptamer makes a point contact with human PDGF-B chain at phenylalanine 84 (with isoleucine at position 83). Because this region is identical in mouse and human PDGF-B chain, high-affinity binding of the aptamer to mouse PDGF-B chain is expected.

As a control aptamer, we used a sequence-scrambled analog of the aptamer conjugated to 40 kD PEG (8). The binding affinity of this scrambled aptamer (NX1976) for PDGF-BB (K_d approximately 1 μM) is 10,000-fold lower compared with the binding affinity of the PDGF-B aptamer (K_d approximately 0.1 nM) (8).

Experimental Model and Experimental Design

All animal studies were approved by the Institutional Review Board. Progressive anti–Thy-1.1 nephritis was induced in 12 male Wistar rats (Charles River, Sulzfeld, Germany), weighing 140 to 160 g at the start of the experiment by a right-sided uninephrectomy. One h later, the rats received a single intravenous bolus injection of 4 mg/kg monoclonal antibody 1-22-3 as described (10). From days 3 to 7 after disease induction, the rats received twice-daily intravenous injections of 5 mg/kg per d PDGF-B aptamer. The lower pole of the left kidney was biopsied on day 8. Because the morphologic changes at this time point of the disease show little interindividual variation (8), only randomly selected rats from each treatment group (see Figure 1) were investigated. After the renal biopsy, the rats remained untreated. Twenty-four-h urine collections were performed every 2 wk until the rats were killed on day 100. BP was measured by tail-cuff plethysmography on day 100. After this, rats were killed and a serum sample as well as renal tissue were collected.

Two uninephrectomized, nephritic control groups were studied. Thirteen rats were treated from days 3 to 7 with 5 mg/kg per d scrambled aptamer while 6 rats received an equivalent volume of phosphate-buffered saline (PBS) twice daily from days 3 to 7. All other experimental parameters were identical to those described above. Food, in particular protein, intake in all nephritic groups was kept at similar levels by pair feeding the rats throughout the study period.

In addition to the three groups described above, two groups of non-nephritic yet uninephrectomized rats were studied. Three rats received the PDGF-B aptamer (5 mg/kg per d from days 3 to 7), and three rats received PBS only. Again, these rats were then followed until they were killed on day 100.

Renal Morphology

Tissue for light microscopy and immunoperoxidase staining was fixed in methyl Carnoy's solution and embedded in paraffin. Four-μm sections were stained with the periodic acid-Schiff reagent and counterstained with hematoxylin. In the periodic acid-Schiff–stained sections, the number of mitoses within 30 to 50 glomerular tufts was determined. Furthermore, in renal sections obtained on day 8, irrevocably injured glomeruli were counted. These were defined as glomeruli in which the whole tuft was still replaced by microaneurysm(s) without evidence of cellular regeneration or completely obsolescent glomeruli. On day 100, the percentage of glomeruli that exhibited focal or global glomerulosclerosis was determined as described previously (11). Tubulointerstitial injury on days 8 and 100 was defined as inflammatory cell infiltrates, tubular dilation and/or atrophy, or interstitial fibrosis. Injury was graded according to Shih *et al.* (12) on a scale of 0 to 4 (0, normal; 0.5, small focal areas of damage; 1, involvement of <10% of the cortex; 2, involvement of 10 to 25% of

Table 1. Effects of treatment with the PDGF-B aptamer on renal immunostaining for TGF-β and on renal cortical TGF-β1 content on day 100 after disease induction in nephritic rats that were treated with the PDGF-B aptamer or scrambled aptamer[a]

Parameter	PDGF-B Aptamer ($n = 6$)	Scrambled Aptamer ($n = 6$)
TGF-β Immunostaining (% of area staining positively)		
glomerular cross sections	29.6 ± 1.1	34.4 ± 1.4
tubulointerstitium (0.09 mm^2)	17.6 ± 0.7	33.9 ± 1.3[b]
Cortical TGF-β1 content (pg/mg protein)	170 ± 21	205 ± 24

[a] PDGF-B, platelet-derived growth factor-B chain; TGF, transforming growth factor.

[b] $P < 0.005$ *versus* nephritic rats that received PDGF-B aptamer.

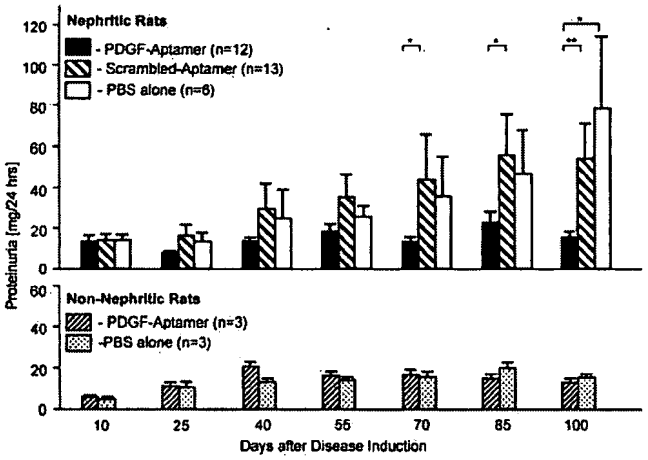

Figure 2. Proteinuria in rats that received the PDGF aptamer, the scrambled aptamer, or PBS alone. *, $P < 0.05$; **, $P < 0.01$.

the cortex; 3, involvement of 25 to 75% of the cortex; 4, extensive damage involving >75% of the cortex). Planar glomerular areas were determined by computerized morphometry (11).

Immunoperoxidase Staining

Four-μm sections of methyl Carnoy's fixed biopsy tissue were processed by a direct or indirect immunoperoxidase technique as described previously (11). Primary antibodies were identical to those described previously (11) and included a murine monoclonal antibody (clone 1A4) to α-smooth muscle actin (α-SMA); a murine monoclonal IgG antibody (clone ED1) to a cytoplasmic antigen present in monocytes, macrophages, and dendritic cells; a murine monoclonal antibody (clone PGF-007) to PDGF-B chain; a murine monoclonal antibody (clone D33) against human muscle desmin; affinity-purified polyclonal goat anti-human/bovine type IV collagen IgG preabsorbed with rat erythrocytes; plus appropriate negative controls. In addition, slides were stained with an affinity-purified polyclonal goat antibody against human type III collagen (Southern Biotechnology, Birmingham, AL). All slides were evaluated by an observer who was unaware of the origin of the slides.

To obtain mean numbers of infiltrating monocytes/macrophages in glomeruli, we evaluated more than 30 consecutive cross sections of glomeruli (range, 30 to 100) and calculated mean values per kidney. To obtain total counts of infiltrating monocytes/macrophages in the renal interstitium, we analyzed more than 40 grid fields (range, 40 to 60), measuring 0.09 mm^2 each, and, again, obtained mean counts per kidney. The stains for α-SMA, types III and IV collagen, PDGF B, and transforming growth factor-β TGF-β (see below) were evaluated using a point-counting method. For this, a grid composed of 121 dots was superimposed on glomeruli (range, 30 to 50; magnification of 100-fold) or 0.09-mm^2 fields of cortical tubulointerstitium (range, 30 to 40) and the percentages of dots overlying stained areas were counted. In the case of immunostaining for desmin, edges of glomerular tufts were scored semiquantitatively depending on the percentage of edge showing positive staining: 0, 0 to 5% stained; I, 5 to 25%; II, 25 to 50%; III, 50 to 75%; IV, >75%.

Electron Microscopy

Maximally 1-mm^3 large tissue pieces fixed in 4% phosphate-buffered formaldehyde were embedded in araldite. Ultrathin sections were stained with lead citrate and viewed in an electron microscope (Zeiss EM10; Zeiss, Oberkochen, Germany). Podocytic foot process width on day 8 was determined in two to five glomeruli of three nephritic rats that were treated with PDGF aptamer and three rats that received scrambled aptamer.

Immunostaining for TGF-β

In randomly selected subgroups of animals (see Table 1), immunostaining for TGF-β was performed on frozen sections fixed in acetone for 5 min. Mean renal functional and histologic changes including SD in the subgroups analyzed were comparable to those of the whole groups (data not shown). A mouse monoclonal antibody against TGF-β1 to -β3 (Genzyme Diagnostics, Cambridge, MA) was used. Bound antibody was detected using an alkaline phosphatase anti-alkaline phosphatase detection system (Dako, Hamburg, Germany). Controls included the omission of the first or second antibody in each section, in which case no staining was observed.

Determination of Renal Cortical TGF-β1 Content

Slices of renal cortex were obtained on day 100 from six nephritic rats that were treated with the PDGF-B aptamer and six nephritic rats that were treated with the scrambled aptamer. Whole cortical tissue was homogenized in 2 ml of Triton X-100 lysis buffer (50 mM HEPES [pH 7.5], 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM ethyleneglycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid, 10% glycerol, 1% Triton X-100, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride) at 4°C. After incubation for 5 min, lysates were centrifuged at 4°C for 15 min at 10,000 × *g*. The TGF-β1 concentrations in the lysates were determined using a commercially available enzyme-linked immunosorbent assay (ELISA; Quantikine TGF-β1; R&D Systems, Wiesbaden, Germany). The protein concentrations in the lysates were determined by the method of Lowry *et al.* (13).

Determination of Renal Collagen Content

It has been shown in rat renal tissue that measurements of hydroxyproline concentration provide a reliable assessment of total

Table 2. Effects of treatment with the PDGF-B aptamer on serum urea concentrations on day 100 after disease induction[a]

Group	Nephritic			Non-Nephritic	
	PDGF-B Aptamer	Scrambled Aptamer	PBS Only	PDGF-B Aptamer	Scrambled Aptamer
Serum urea (mmol/L)	8.0 ± 0.3	11.8 ± 1.5	11.0 ± 1.2[b]	7.5 ± 0.4	7.9 ± 0.1
Creatinine clearance (ml/min)	1.46 ± 0.07	1.13 ± 0.11[b]	1.33 ± 0.13	1.52 ± 0.06	1.55 ± 0.04

[a] PBS, phosphate-buffered saline.
[b] $P < 0.05$ *versus* nephritic rats that received PDGF-B aptamer.

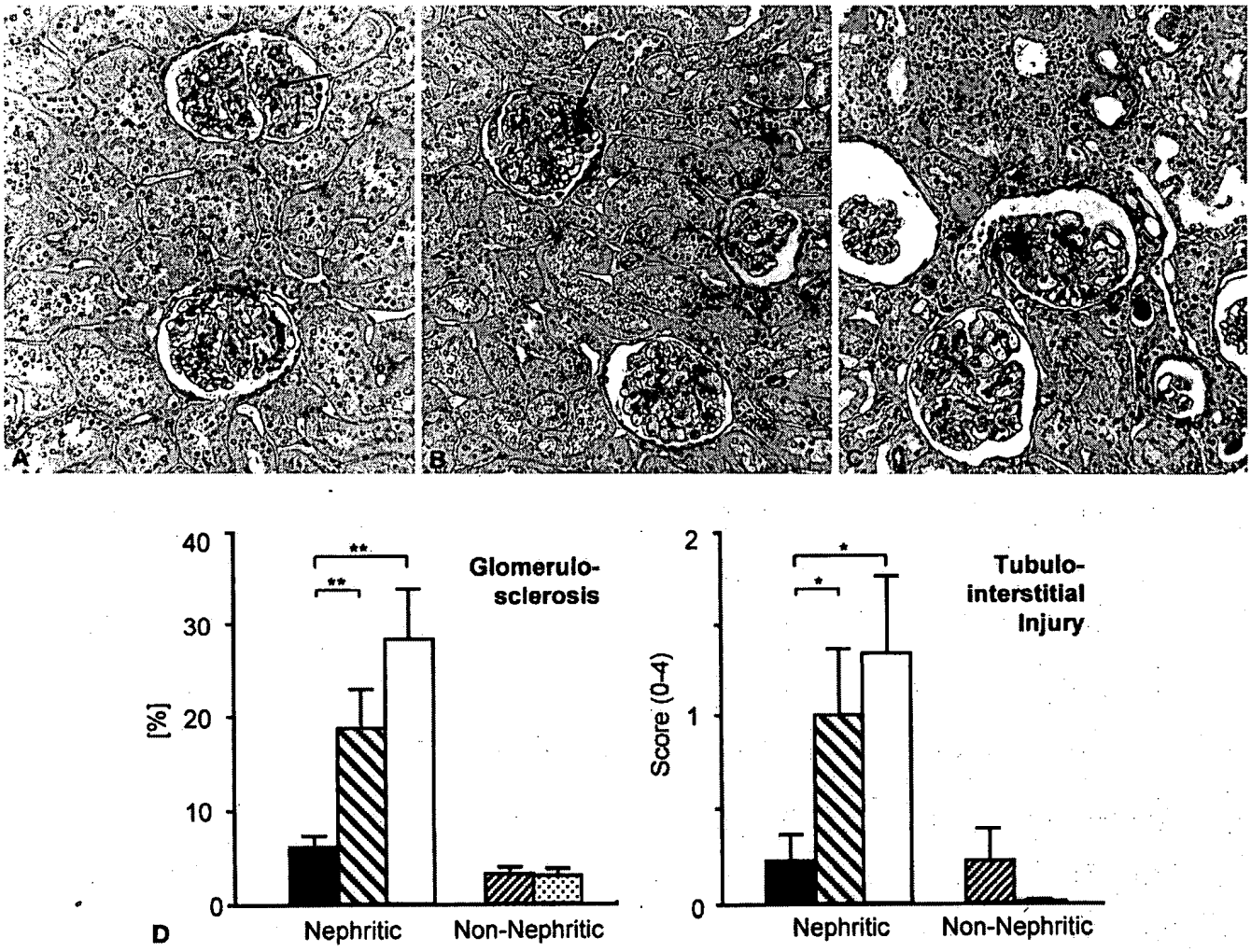

Figure 3. Renal light microscopic changes on day 100 after disease induction. (A) Periodic acid-Schiff–stained renal section of a rat that received PDGF aptamer. Residual mesangial hypercellularity and matrix expansion is present in occasional glomeruli (arrow), whereas no focal segmental glomerulosclerosis or tubulointerstitial damage is noted. Findings in rats that received the scrambled aptamer ranged from occasional focal segmental glomerulosclerosis (arrow) with relative preservation of the tubulointerstitium (B) to widespread focal segmental glomerulosclerosis and tubulointerstitial damage (C), (D) Quantitative assessment of focal segmental glomerulosclerosis and tubulointerstitial changes (see Materials and Methods section for details). *, $P < 0.05$; **, $P < 0.01$. Magnification, ×200 in A, B, and C.

collagen content (14). Renal cortical hydroxyproline concentrations therefore were determined using previously described methods (14,15). Renal collagen content was expressed as μg per mg protein as determined by the method of Lowry *et al.* (13).

Miscellaneous Measurements

Urinary protein was measured using the Bio-Rad Protein Assay (Bio-Rad Laboratories GmbH, Munich, Germany) and bovine serum albumin (Sigma, Deisenhofen, Germany) as a standard. Serum urea concentration was measured using an autoanalyzer (Beckman Instruments GmbH, München, Germany).

Statistical Analyses

All values are expressed as mean ± SEM. Statistical significance (defined as $P < 0.05$) was evaluated using Kruskal-Wallis tests or Mann-Whitney *U* tests.

Results

Acute Effects of the PDGF-B Aptamer in Rats with Anti–Thy-1.1 Nephritis

In renal biopsies obtained from nephritic rats on day 8 after disease induction, treatment with the PDGF-B aptamer significantly reduced the number of glomerular mitoses in comparison with PBS-treated rats (Figure 1). This reduction seemed to be due largely to reduced mesangial cell proliferation as suggested by our previous data (8) and by the finding that the PDGF-B aptamer significantly ameliorated the glomerular *de novo* expression of α-SMA (Figure 1), a specific marker of mesangial cell activation (16). Treatment with the PDGF-B aptamer also reduced the glomerular accumulation of type IV collagen and the influx of monocytes/macrophages (Figure 1). In all cases, treatment with the scrambled aptamer had no effect on the morphologic changes (Figure 1). Finally, the

number of irrevocably injured glomeruli was significantly lower in nephritic rats that received the PDGF-B aptamer (4.4 ± 0.8% *versus* 18.1 ± 4.4% in those that received scrambled aptamer and 13.9 ± 4.0% in those that received PBS; $P < 0.01$ *versus* PDGF-B aptamer group in both cases).

Both nephritic rats that received the PDGF-B aptamer and those that received the scrambled aptamer were normotensive on day 4, and no significant difference was noted between the groups (data not shown).

With the exception of glomerular α-SMA, treatment with the PDGF-B aptamer lowered all parameters investigated in nephritic rats to levels comparable to those observed in PBS-treated non-nephritic rats (Figure 1). Mild focal tubulointerstitial injury was present in some nephritic rats but was not affected by the different treatment modalities (Figure 1).

Chronic Effects of the PDGF-B Aptamer in Rats with Anti–Thy-1.1 Nephritis

Proteinuria, BP, and Renal Function. As shown in Figure 2, treatment of nephritic rats with the PDGF-B aptamer from days 3 to 7 prevented the subsequent development of proteinuria, whereas treatment with the scrambled aptamer was ineffective. Levels of proteinuria observed in the nephritic PDGF-B aptamer group were indistinguishable from those observed in non-nephritic rats. BP in nephritic rats that received the PDGF-B aptamer were normal on day 100 (101 ± 5 mmHg) and lower than in the nephritic rats that received scrambled aptamer (115 ± 10 mmHg), although statistical significance was not reached. Serum urea concentrations were increased and creatinine clearances decreased in the nephritic groups that received PBS or scrambled aptamer, whereas those of the PDGF-B aptamer groups were indistinguishable from non-nephritic rats (Table 2).

Glomerulosclerosis and Tubulointerstitial Damage. Light microscopic changes, *i.e.*, the extent of focal segmental glomerulosclerosis and tubulointerstitial damage, were significantly reduced on day 100 in nephritic rats that were treated with the PDGF-B aptamer as compared with those that received scrambled aptamer or PBS alone (Figure 3). Chronic changes in non-nephritic rats were mild and their extent was comparable to those observed in nephritic rats that received the PDGF-B aptamer (Figure 3D).

To investigate potential mechanisms by which the PDGF-B aptamer reduced the frequency of glomerulosclerosis, we assessed glomerular hypertrophy via the measurement of the cross-sectional glomerular area and podocyte damage via their *de novo* expression of the cytoskeletal protein desmin (17). As shown in Figure 4, in nephritic rats that received the PDGF-B aptamer, both of these parameters were reduced to levels observed in non-nephritic rats on days 8 and 100 of the study period. By electron microscopy, no areas of foot process fusion or detachment from the glomerular basement membrane were noted. However, foot process width in nephritic rats that were treated with PDGF-B aptamer was reduced as compared with those that received scrambled aptamer (0.64 ± 0.03 μm *versus* 0.77 ± 0.14 μm; $n = 3$ each). Glomerular counts of mitoses and α-SMA staining scores on day 100 were low and not

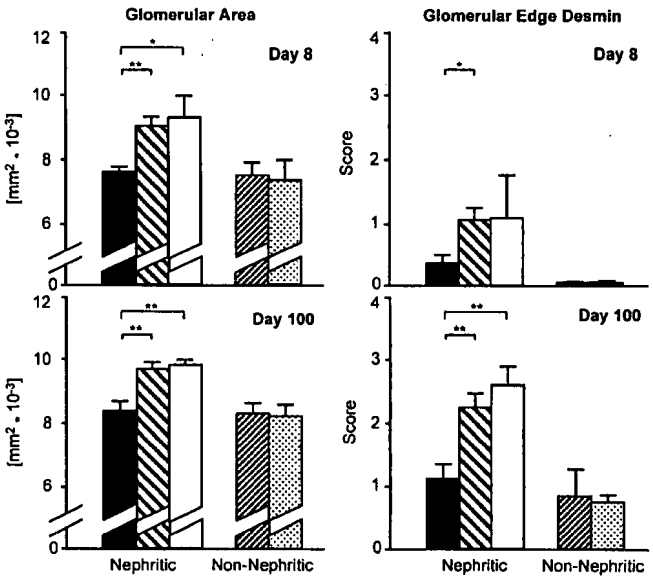

Figure 4. Glomerular planar area and expression of desmin on days 8 and 100 after disease induction in rats that received the PDGF aptamer, the scrambled aptamer, or PBS alone. *, $P < 0.05$; **, $P < 0.01$.

significantly different in all nephritic groups (data not shown). Furthermore, the glomerular PDGF-B staining scores on day 100 in the aptamer-treated group were low (0.68 ± 0.04; $n = 12$) and not significantly different from the scrambled aptamer-treated rats (0.61 ± 0.04; $n = 13$).

Renal Extracellular Matrix Accumulation. Renal matrix accumulation was assessed by immunostaining for basement membrane, *i.e.*, type IV, and interstitial type, *i.e.*, type III, collagen. In nephritic rats, treatment with the PDGF-B aptamer reduced the accumulation of both collagen types to similar levels as those observed in non-nephritic controls (Figure 5). Furthermore, the total renal collagen content, as assessed via the analysis of hydroxyproline content, was also decreased in PDGF-B aptamer-treated nephritic rats in comparison to the other nephritic groups (Table 3).

The renal expression of the profibrotic cytokine TGF-β on day 100 after disease induction was increased in rats that received the scrambled aptamer or PBS. Immunoreactivity was present in the expanded interstitium, some infiltrating cells, some tubular cells, and glomerular cells and/or extracellular matrix (Figure 6B). TGF-β immunostaining was mostly decreased in globally sclerosed glomeruli. In rats that were treated with the PDGF aptamer, the staining pattern was comparable to that observed in normal rats in that some glomerular cells, very few tubular cells, the renal interstitium, and occasional infiltrating cells stained positively (Figure 6A). Quantitative evaluation of the renal cortical TGF-β expression confirmed the marked reduction in PDGF aptamer-treated rats, in particular in the renal tubulointerstitium (Table 1). Specific determination of the whole cortical TGF-β1 content by ELISA revealed a lesser content in PDGF aptamer-treated rats as compared with

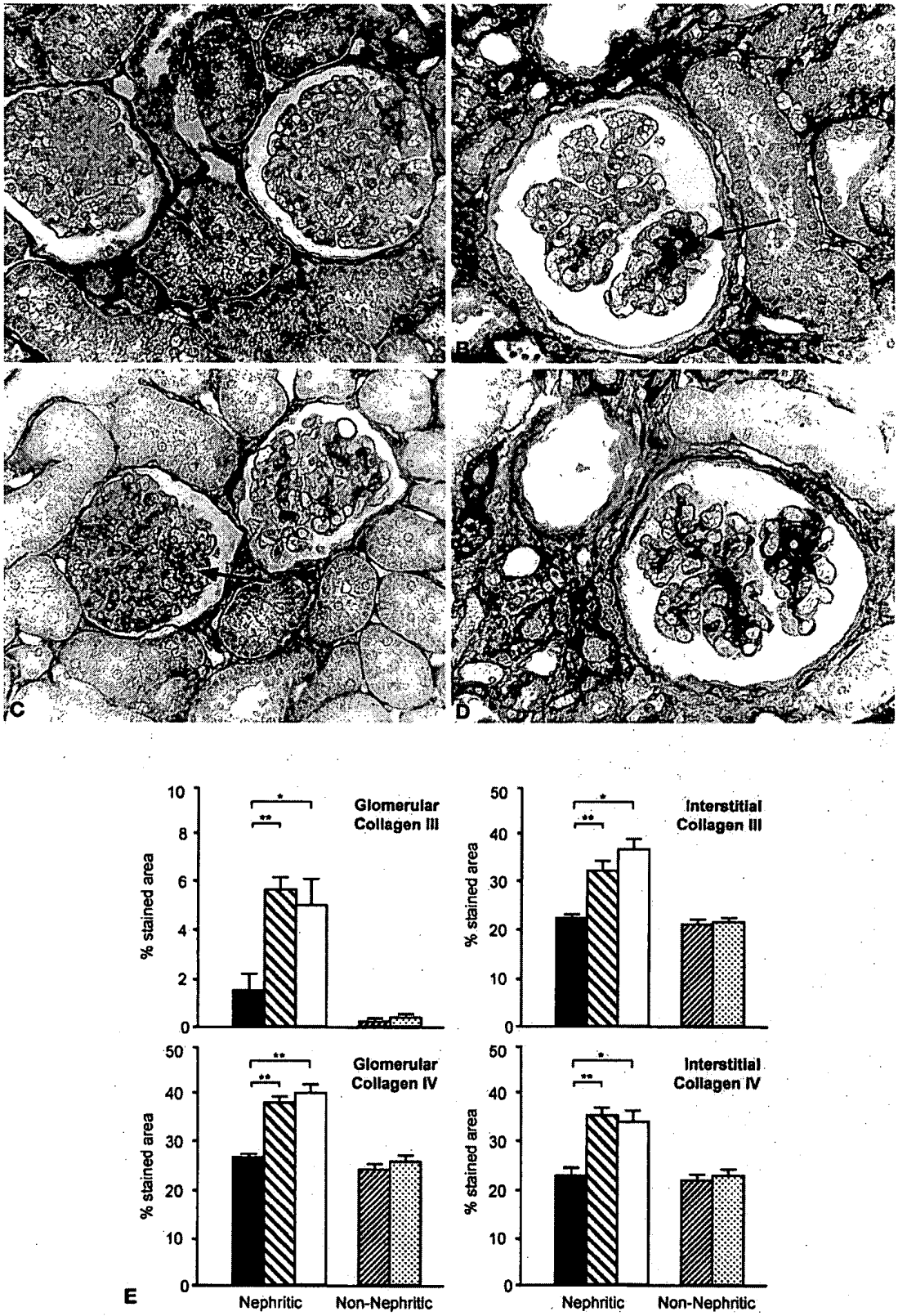

Figure 5. Renal expression of type III and IV collagen on day 100 after disease induction. (A) Renal type III collagen expression in a rat that received PDGF aptamer is confined to interstitial areas. (B) Type III collagen in a scrambled aptamer-treated rat is present in some glomeruli (arrow) and the widened interstitium. (C) Type IV collagen expression in a PDGF aptamer-treated rat shows a normal glomerular and interstitial distribution except for occasional mesangial matrix expansion (arrow). (D) Type IV collagen expression in a scrambled aptamer-treated rat is increased in both glomeruli and the renal interstitium. (E) Quantitative evaluation of renal type III and IV collagen immunostaining. *, $P < 0.05$; **, $P < 0.01$.

Table 3. Effects of treatment with the PDGF-B aptamer on renal collagen content on day 100 after disease induction

Group	Nephritic		
	PDGF-B Aptamer ($n = 6$)	Scrambled Aptamer ($n = 6$)	PBS Only ($n = 3$)
Collagen content (μg/mg protein)	79.9 ± 3.5	121.4 ± 5.4[a]	94.5 ± 12.3

[a] $P < 0.01$ *versus* nephritic rats that received PDGF-B aptamer.

those that received scrambled aptamer, which, however, failed to reach statistical significance (Table 1). These data suggest that TGF-β2 and/or -β3 overexpression, which was not detected in the TGF-β1–specific ELISA, might also have contributed to the increased renal TGF-β immunostaining in rats that received scrambled aptamer.

Renal Monocyte/Macrophage Infiltration. Figure 7 shows that glomerular monocyte/macrophage counts on day 100 after disease induction were low and in a comparable range in all nephritic groups. In contrast, renal interstitial monocyte/macrophage counts were increased in nephritic rats that received scrambled aptamer or PBS in comparison with those that received PDGF-B aptamer.

Discussion

In the present study, we first established that under our experimental conditions the PDGF-B aptamer was as potent in reducing acute mesangioproliferative changes as in our previous study (8), in which a different nephritis model had been investigated (reversible anti–Thy-1.1 nephritis induced by monoclonal antibody OX7 in rats with two kidneys). The data show that in both models, the PDGF-B aptamer led to comparable reductions of mesangioproliferative changes and glomerular monocyte/macrophage influx. Because it is well established that the acute phase in the anti–Thy-1.1 nephritis model is PDGF dependent (8), these data, in concert with our recent *in vitro* data (8) as well as recent data in the PDGF-dependent carotid restenosis model (18), substantiate the notion that the aptamer acted *in vivo* by specifically antagonizing PDGF-B chain.

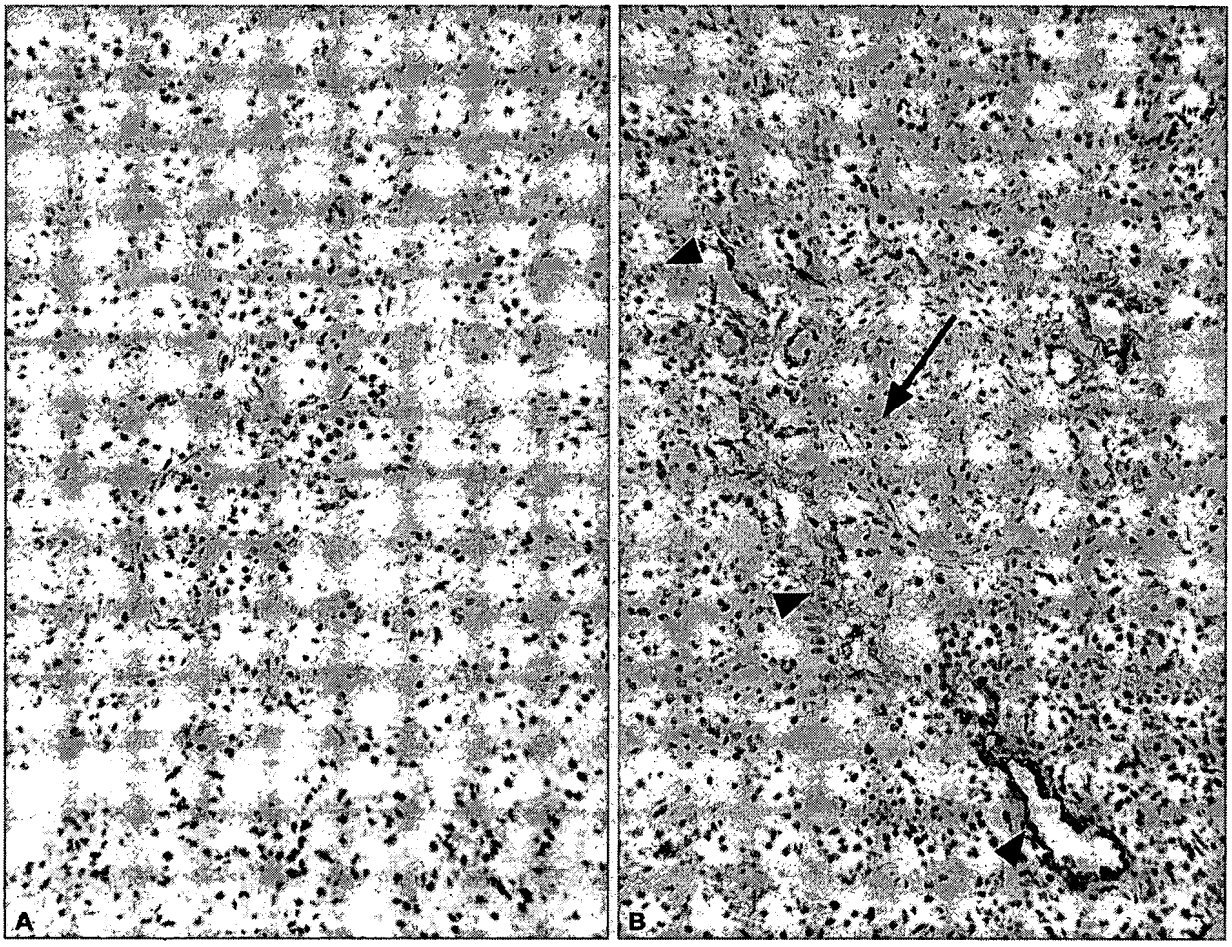

Figure 6. Renal expression of transforming growth factor-β (TGF-β) on day 100 after disease induction. (A) Renal TGF-β expression in a rat that received PDGF aptamer is largely confined to interstitial areas. (B) TGF-β expression in a scrambled aptamer-treated rat is present in a glomerulus (arrow), damaged tubular cells, the widened interstitium, and interstitial infiltrates (arrowhead).

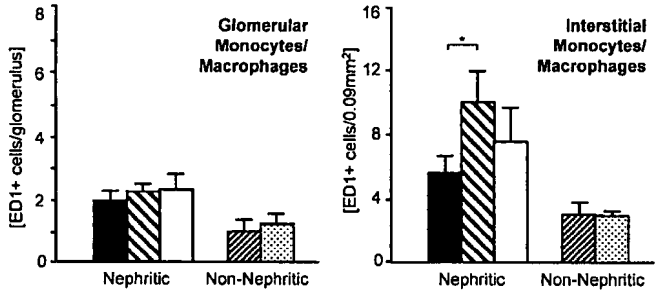

Figure 7. Glomerular and tubulointerstitial monocyte/macrophage counts on day 100 in rats that received the PDGF aptamer, the scrambled aptamer, or PBS alone. *, $P < 0.05$; **, $P < 0.01$.

We next asked whether the acute amelioration of mesangioproliferative changes would extend to the prevention of progressive renal damage. Because some evidence is available to invoke PDGF-B chain in the pathogenesis of renal interstitial fibrosis (19), we confined the period of PDGF antagonism to the phase of acute glomerular damage to avoid therapeutic effects on secondary pathomechanisms of renal failure, such as interstitial fibrosis. The central finding of the present study was that transient yet potent inhibition of PDGF-B chain during the acute phase of mesangioproliferative nephritis almost completely prevented the subsequent development of proteinuria, glomerulosclerosis, tubulointerstitial damage, and renal fibrosis. These observations provide a strong argument against concerns that inhibition of overshooting mesangial cell growth and matrix production after injury might represent inhibition of healing and thus aggravate glomerular damage. Rather, our findings place the mesangial overexpression of PDGF-B chain at a very early and possibly key position in the cascade of events that eventually leads to renal failure in diseases associated with mesangioproliferative changes.

By which mechanism(s) did the transient inhibition of PDGF exert beneficial long-term effects? PDGF antagonism was unlikely to have affected the extent of early mesangial damage, because treatment was started 3 d after disease induction. At this time point, immune injury in the anti–Thy-1.1 nephritis model is already past its maximum (8,20). Treatment-related effects on other important variables that contribute to progression of renal damage, such as systemic hypertension or dietary protein intake (21–23), also are unlikely because normotension was maintained during the phase of PDGF antagonism and because pair feeding was performed throughout the study period. It also seems unlikely that the PDGF-B aptamer acted directly on tubular cells after its glomerular filtration, because in the acute phase of the anti–Thy-1.1 nephritis, no tubular overexpression of PDGF-B has been noted (4,5). Finally, on day 100, no long-term effects of PDGF-B aptamer treatment on glomerular cell proliferation, mesangial cell activation, or glomerular PDGF expression persisted. This suggests that ongoing mesangial cell activation and proliferation are not required for the development of progressive renal damage.

Possibly the most important consequence of the treatment with PDGF-B aptamers was the reduction of early glomerular hypertrophy. This likely resulted from a direct antiproliferative effect of the PDGF-B aptamer on mesangial cells (8) as well as from antifibrotic actions of PDGF antagonism (reviewed in references 4 and 5). How does reduction of glomerular hypertrophy relate to the prevention of renal failure and progressive structural damage? Glomerular hypertrophy has been recognized as a strong predictor for progressive renal disease in both experimental and human studies (2,24). We recently showed that glomerular hypertrophy is associated closely with podocyte damage (11), which is regarded as a crucial event in the development of glomerulosclerosis (25). To investigate whether PDGF antagonism in the present study also affected podocyte damage, we assessed the podocytic *de novo* expression of desmin, a marker of podocyte damage (17), and electron microscopic podocyte changes. These data showed that PDGF antagonism in the current nephritis model reduced not only mesangioproliferative changes but also the associated (secondary?) podocyte as well as irreversible glomerular damage. The effect of PDGF-B antagonism on podocyte damage was probably an indirect one, because podocytes do not express PDGF receptors *in vivo* and glomerular epithelial cells *in vitro* do not respond to even high PDGF-B concentrations (26–31). Apart from its role in the pathogenesis of glomerulosclerosis, podocyte damage is also a central mechanism in the development of proteinuria (32). Our observation that treatment with the PDGF-B aptamer prevented the development of progressive proteinuria therefore provides further, albeit indirect, evidence for a treatment-related decrease in podocyte damage. Proteinuria in turn is viewed as an important determinant of tubulointerstitial damage, which is associated strongly with progressive renal failure (33). Therefore, by reducing early glomerular hypertrophy and podocyte damage, transient antagonism of PDGF may have effected the various beneficial long-term effects noted in the present study.

One mechanism by which the prevention of progressive proteinuria in the PDGF aptamer-treated nephritic rats translated into reduced renal scarring may involve the action of TGF-β. In the kidney, TGF-β has been established to be of central importance in the mediation of fibrotic changes (34,35). Our observation that transient PDGF antagonism *in vivo* resulted in a long-term reduction of tubulointerstitial TGF-β expression thus provides a molecular basis by which interstitial fibrosis was reduced. Whereas in glomerular mesangial cells a direct link between the expression of PDGF-B chain and subsequent TGF-β induction has been established (36), the relationship between antagonism of PDGF-B chain and reduced tubulointerstitial TGF-β expression seems to be indirect. Thus, it is likely that, analogous to observations in other models of renal interstitial damage, the tubulointerstitial TGF-β overexpression resulted from proteinuria-induced activation of renal tubular cells and the subsequent induction of peritubular inflammatory changes (37). Alternatively, it is conceivable that the tubulointerstitial overexpression of TGF-β was mediated by mechanisms different from proteinuria, such as the leakage of proinflammatory substances from inflamed glomeruli via the urine or through the glomerular stalk.

In conclusion, these data identify PDGF-B chain as a growth factor for which transient, specific antagonism prevents the development of progressive renal scarring. In this respect, the role of PDGF-B chain seems more central than in vascular disease, where the same aptamer potently suppressed the formation of a neointima in the rat carotid restenosis model yet had a smaller long-term protective effect after the cessation of treatment (18). A possible correlate for these observations is provided by mice that are genetically deficient for PDGF-B chain, which develop no mesangium while the gross morphology of vascular smooth muscle cells is normal (38). The present data therefore suggest that antagonism of PDGF may be a useful therapeutic approach to renal diseases characterized by mesangioproliferative changes. PDGF-B chain is a particularly attractive therapeutic target, because a transgenic mouse line, which expresses high levels of circulating PDGF antagonist during adult life only, does not exhibit phenotypic abnormalities (39). This suggests that it may be safe to block PDGF-B chain in adult life, where it does not seem to have central physiologic roles.

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PDGF signal transduction inhibition ameliorates experimental mesangial proliferative glomerulonephritis[1]

RICHARD E. GILBERT, DARREN J. KELLY, TARA MCKAY, STEVEN CHADBAN, PRUDENCE A. HILL, MARK E. COOPER, ROBERT C. ATKINS, and DAVID J. NIKOLIC-PATERSON

Department of Medicine, University of Melbourne, St. Vincent's Hospital; Department of Nephrology, Monash Medical Center and Department of Medicine, Monash University; Department of Anatomy and Cell Biology, University of Melbourne; Department of Medicine, University of Melbourne, Austin and Repatriation Medical Center, Melbourne, Victoria, Australia

PDGF signal transduction inhibition ameliorates experimental mesangial proliferative glomerulonephritis.

Background. Platelet-derived growth factor (PDGF) has been consistently implicated in the cell proliferation and extracellular matrix accumulation, which characterize progressive glomerular disease. In the present study, the effects of a potent and selective inhibitor of PDGF receptor tyrosine kinase, STI 571, were examined in vitro and in vivo.

Methods. Cultured mesangial cells were incubated with PDGF (50 ng/mL) and fibroblast growth factor-2 (FGF-2; 50 ng/mL) and treated with STI 571 (0.13 to 2.0 μmol/L). Experimental mesangial proliferative glomerulonephritis was induced in male Wistar rats with monoclonal OX-7, anti-rat Thy-1.1 antibody with rats randomized to receive either STI 571 (50 mg/kg intraperitoneally daily) or vehicle. Animals were examined six days later.

Results. In vitro, both PDGF and FGF-2 induced a threefold increase in mesangial cell ^{3}H-thymidine incorporation. STI 571 reduced PDGF but not FGF-2–stimulated mesangial cell proliferation in a dose-dependent manner, with complete abolition at 0.4 μmol/L. In animals with Thy-1.1 glomerulonephritis, PDGF receptor tyrosine kinase blockade was associated with significant reductions in mesangial cell proliferation ($P < 0.001$), the number of activated (α-smooth muscle positive) mesangial cells, and glomerular type IV collagen deposition ($P < 0.001$).

Conclusion. The amelioration of the pathological findings of experimental mesangial proliferative glomerulonephritis by blockade of PDGF receptor activity suggests the potential clinical utility of this approach as a therapeutic strategy in glomerular disease.

Cellular proliferation and extracellular matrix accumulation are characteristic features of progressive glomerular diseases in humans, a major cause of end-stage renal failure throughout much of the world. The mechanisms underlying these changes are incompletely understood, although studies conducted over the past 15 years suggest a key role for growth factors in the pathogenesis of renal injury [1]. While many act in a pleiotropic fashion, increasing evidence suggests that the effects of specific growth factors predominate in certain physiological and pathological situations [2–5].

Platelet-derived growth factor (PDGF) has been widely implicated in the pathogenesis of progressive renal injury in both human disease and experimental models [6]. PDGF is synthesized by resident renal cells [7, 8] and by infiltrating macrophages [8] that are frequently associated with progressive renal injury [9]. The actions of PDGF include stimulation of mesangial cell proliferation [10], increased extracellular matrix (ECM) synthesis [11], and increased expression of the prosclerotic cytokine, transforming growth factor-β (TGF-β) [12]. Furthermore, PDGF levels are also increased in response to a variety of factors that have been implicated in renal disease, including angiotensin II [13], endothelin [14], inflammatory cytokines [15], and advanced glycation end products [11, 12].

Specific inhibition of PDGF action is therefore a major target for therapy in glomerular disease. Recently, selective, receptor tyrosine kinase (RTK) inhibitors with in vivo activity have been synthesized. Of this class of 2-phenylaminopyridines, signal transduction inhibitor (STI) 571 is a potent and selective inhibitor of PDGF RTK and v-Abl kinase (with which it shares substantial homology) [16]. Such agents may therefore provide therapeutic potential in the treatment of disorders characterized by overexpression of PDGF or v-Abl such as glomerular disease and chronic myeloid leukemia (CML).

The present study sought first to examine the effects of PDGF-RTK inhibition with STI 571 on mesangial cell proliferation in vitro, and also to determine the efficacy

[1] See Editorial by Floege and Ostendorf, p. 1592

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of this approach on the pathological hallmarks of experimental mesangial proliferative nephritis, mesangial proliferation, and matrix accumulation.

METHODS

Drug preparation

STI 571, the properties of which have been previously described in detail elsewhere [16], was obtained from Novartis Pharmaceuticals (generous gift of Dr. E. Buchdunger, Novartis, Basel, Switzerland). For in vitro studies, STI 571 was diluted to 10 mmol/L in dimethyl sulfoxide (DMSO). This stock solution was then diluted in cell culture medium to a final concentration of 0.013 to 2.0 μmol/L. For in vivo studies, fresh solution was prepared daily by dissolving the compound in DMSO (200 mg/mL) and diluting this stock solution 1:10 in normal saline.

Antibodies

Monoclonal OX-7, anti-rat Thy-1.1 antibody was used for the induction of mesangial proliferative glomerulonephritis [17]; macrophages were detected using ED1, anti-rat CD68 [18]. Myofibrobalsts were identified by labeling with 1A4, anti-human α-smooth muscle actin (Sigma Immunochemicals, St. Louis, MO, USA), and M744, anti-BrdU (Dako, Glostrup, Denmark) was used to identify proliferating cells. A polyclonal goat anti-bovine/anti-human type IV collagen antibody (Southern Biotechnology, Birmingham, AL, USA) was also used to examine extracellular matrix.

Mesangial proliferation

A well-characterized cloned mesangial cell line (1097) isolated from Sprague-Dawley rats [19] was used between passages 20 and 30. For these experiments, cells were cultured in RPMI 1640 Medium (GIBCO, Grand Island, NY, USA) with heat-inactivated fetal calf serum (FCS), 100 U/mL penicillin, and 100 μg/mL streptomycin in humidified 5% CO_2 atmosphere at 37°C. Mesangial cells were plated out at low density in 96-well flat-bottomed microtiter plates in RPMI/10% FCS and allowed to adhere overnight. The subconfluent cells were then starved for three days in RPMI/0.5% FCS. A 48-hour proliferation assay was then performed. STI 571 0.013 to 2.0 μmol/L or drug diluent was then added to cells in RPMI/0.5% FCS followed in 30 minutes by the addition of either recombinant PDGF-BB 50 ng/mL (Boehringer-Mannheim, Mannheim, Germany) or recombinant fibroblast growth factor-2 (FGF-2; Calbiochem, La Jolla, CA, USA) 50 ng/mL. During the last six hours of culture, 0.5 μCi/well ^{3}H-thymidine was added. Cells were then harvested by removing the medium, washing twice in warm phosphate-buffered saline (PBS), and dissolving in 100 μL 0.2 mol/L sodium hydroxide. This solution was then neutralized with hydrochloric acid, and scintillation counting was performed with a β counter (Wallace Rackbeta, Wallac Oy, Turku, Finland). Replicates of six wells were used.

Rat anti–Thy-1 nephritis

Male Wistar rats (150 to 170 g) were obtained from Monash Animal Services (Melbourne, Australia). Anti–Thy-1 nephritis was induced in two groups of eight rats by an intravenous injection of 5 mg/kg OX-7 IgG, as previously described [20]. Starting one day after OX-7 IgG administration, animals received daily intraperitoneal injections with either STI 571 (50 mg/kg) or vehicle control (10% DMSO in saline) until killed on day 6, the peak of mesangial proliferation in this model [20]. The dose of 50 mg/kg/day was selected on the basis of previous studies on the inhibition of tumor growth [16]. The administration of STI 571 was commenced 24 hours after OX-7 administration as mesangiolysis is complete by this time [20]. Three hours prior to sacrifice, all rats were given an intraperitoneal injection of 50 mg/kg bromodeoxyuridine (BrdU) in order to label cells in the DNA synthetic (S) phase of the cell cycle. A group of eight normal rats was also injected with BrdU three hours before sacrifice.

Proteinuria and renal function assessment

Twenty-four hour urine collections and blood samples were taken on day −3 (prior to experiment) and day 6. Urinary protein concentration was measured by the benzethonium chloride method [21]. Serum and urine creatinine levels were measured using the Jaffé rate reaction [22].

Histology

Tissues were fixed in 10% neutral-buffered formalin and were embedded in paraffin. Kidney sections (4 μm) were stained with periodic acid-Schiff's reagent (PAS). Quantitation of nuclei was performed by examining 50 hilar glomeruli per animal.

Immunohistochemistry

Type IV collagen. Immunostaining for type IV collagen was performed as previously described [23]. In brief, kidney sections were rehydrated and treated with 1% H_2O_2/methanol followed by incubation in Protein Blocking Agent (Lipshaw-Immunon, Pittsburgh, PA, USA) for 20 minutes at room temperature. Sections were then incubated with type IV collagen antibody for 60 minutes at room temperature, washed in PBS, and incubated with biotinylated goat anti-rabbit immunoglobulin (Dako, Carpinteria, CA, USA) followed by incubation with avidin-biotin peroxidase complex (ABC; Vector, Burlingham, CA, USA). Peroxidase conjugates were subsequently localized using diaminobenzidine tetrahydrochloride (DAB)

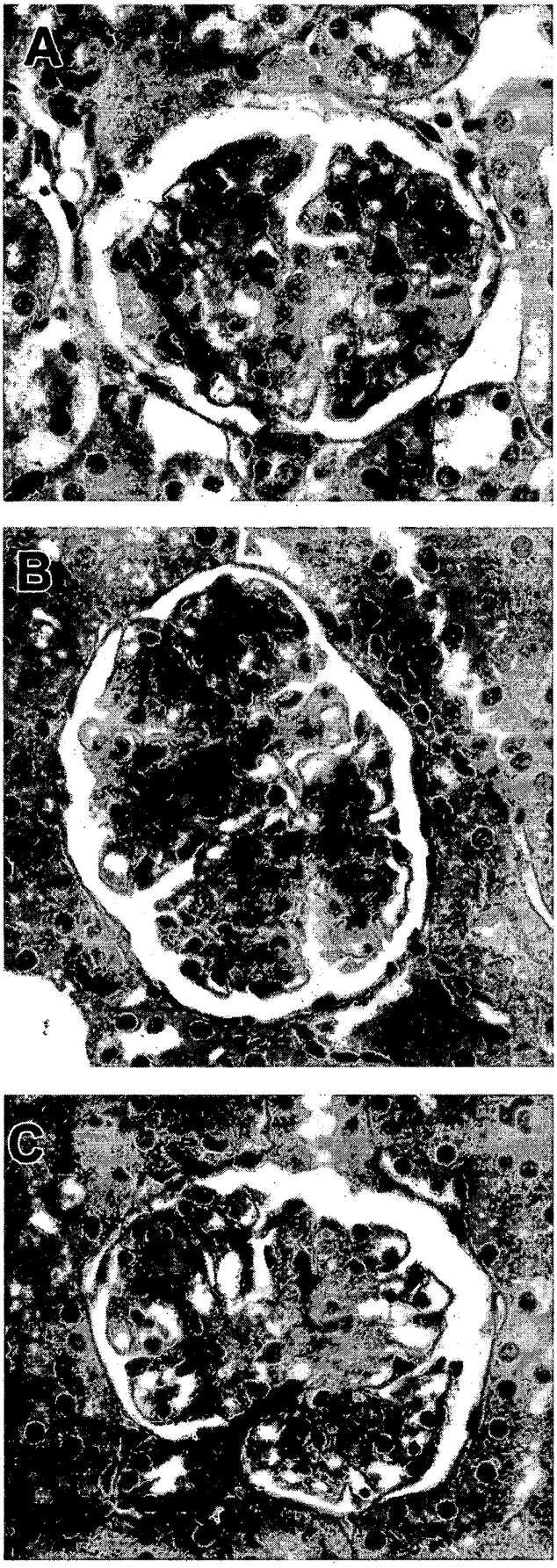

Fig. 2

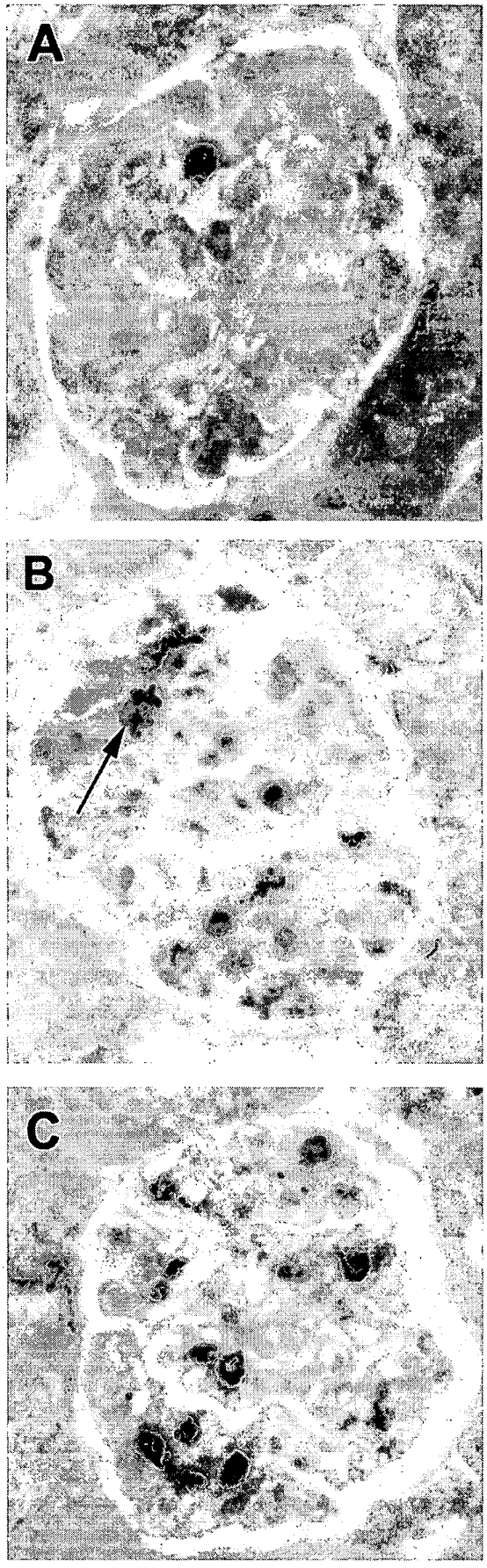

Fig. 4

Fig. 2. Photomicrograph of PAS-stained kidney section from control animals (*A*), rats with experimental mesangial proliferative glomerulonephritis receiving either vehicle (*B*) or STI 571 (*C*). Magnification ×360. Compared with control animals, untreated rats show marked glomerular hypercellularity and mesangial matrix expansion. STI 571–treated rats show near normal glomerular histology. Reproduction of this figure in color was made possible by a grant from Novartis, Australia.

Fig. 4. Effects of STI 571 on mesangial cell proliferation as assessed by double immunostaining with BrdU (blue color) and ED1 (brown color). Photomicrograph showing macrophages (ED 1+, brown), proliferating macrophages (BrdU+/ED 1+ cells, blue and brown) and proliferating mesangial cells (BrdU+/ED 1− cells, blue) in control animals (*A*) and rats with experimental mesangial proliferative glomerulonephritis receiving either vehicle (*B*) or STI 571 (*C*) treatment was associated with reduced mesangial cell proliferation. Magnification ×360. Reproduction of this figure in color was made possible by a grant from Novartis, Australia.

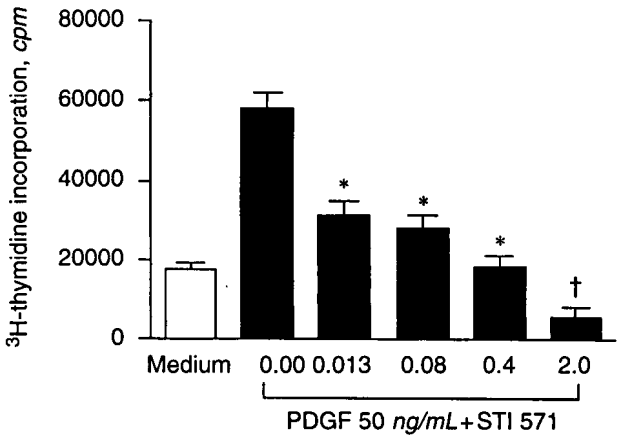

Fig. 1. Effects of STI 571 on platelet-derived growth factor (PDGF)-stimulated DNA synthesis. Data are expressed as mean ± SD. $*P <$ 0.001 vs. PDGF (50 ng/mL) without STI 571. $\dagger P < 0.001$ vs. medium.

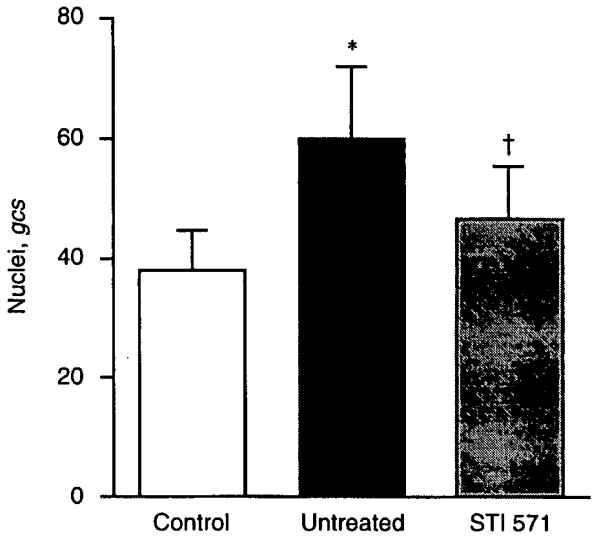

Fig. 3. Glomerular cellularity as assessed by the number of nuclei (mean ± SD) per glomerular cross-section (gcs) in 50 hilar glomeruli per animal. Glomerular hypercellularity was significantly attenuated by STI 571. $*P < 0.05$ vs. control; $\dagger P < 0.05$ vs. untreated.

as a chromogen. Sections were then counterstained with Mayer's hematoxylin.

α-Smooth muscle actin. Immunostaining for α-smooth muscle actin was performed in formalin fixed tissue sections using a microwave-based technique to prevent antibody cross-reactivity, as previously described [24]. In brief, sections were microwave treated for 10 minutes in 0.01 mol/L sodium citrate buffer, pH 6.0, and then labeled with α-smooth muscle actin antibody using a three-layer peroxidase-antiperoxidase method and developed with 3,3-DAB (Sigma) to produce a brown color.

ED 1 and BrdU: Double staining. Double immunohistochemical staining was performed in formalin-fixed tissue sections using a microwave-based technique to prevent antibody cross-reactivity, as previously described [24]. In brief, sections were labeled with ED 1 as described above for α-smooth muscle actin. Sections were then microwave treated for a second time, labeled at 4°C with M744 anti-BrdU antibody using the three-layer phosphatase-antiphosphatase method, and developed with fast blue BB salt (Ajax Chemicals, Melbourne, Australia) to produce a blue color. Sections had a weak counterstain with PAS and mounted in aqueous medium.

Negative controls. Sections incubated with protein blocking agent instead of primary antisera served as negative controls. Tissues were also incubated with irrelevant isotype control antibodies as previously described [24]. Tissues treated in this manner showed no positive staining.

Quantitation of immunohistochemistry

Sections double stained with BrdU and ED 1 antibody were used to quantitate for ED 1+BrdU+, ED 1+ BrdU−, and ED 1−BrdU+ cells. Fifty hilar glomeruli were scored under high power (×400), and the different populations were expressed as the mean ± SD per glomerular cross-section. As podocytes do not proliferate and glomerular endothelial cells account for <3% of proliferating cells in this OX-7–induced disease model [24], proliferating cells were identified as either macrophages (ED 1+BrdU+) or mesangial cells (ED 1− BrdU+) as previously described [24]. All scoring was performed with the observer masked to the study group.

The magnitude of immunostaining for α-smooth muscle actin or type IV collagen was quantitated using computer-assisted image analysis as previously described [25, 26]. In brief, for each tissue section, images from

three nonoverlapping, randomly selected fields were examined by light microscopy (Olympus BX-50; Olympus Optical, Tokyo, Japan) and digitized using a high-resolution camera (Fujix HC-2000; Fujifilm, Tokyo, Japan). All images were obtained using a ×20 objective lens. Digitized images were then captured on a Power Macintosh G3 computer (Apple Computer Inc., Cupertino, CA, USA) equipped with an in-built graphic board and opened using analytical software (Analytical Imaging Software, Ontario, Canada).

The area of brown on an immunoperoxidase-stained section was selected for its color range, and the proportional area of tissue with this range of color was then quantitated on 50 hilar glomeruli per animal such that the magnitude of immunolabeling was expressed as the proportional area of the tissue section that stained brown.

Statistics

All data are shown as mean ± SE unless otherwise specified. Data were analyzed by analysis of variance (ANOVA) using the StatView IV program (Brainpower, Calabasas, CA, USA) on a Macintosh G3. Comparisons between group means were performed by Fisher's least significant difference method. A P value of less than 0.05 was considered statistically significant.

RESULTS

PDGF-induced mesangial cell proliferation in vitro

The ability of PDGF to stimulate proliferation in serum-starved mesangial cells was inhibited by pretreatment of cells with STI 571 in a dose-dependent fashion (Fig. 1). Complete inhibition of PDGF-induced mesangial ^{3}H-thymidine incorporation was seen at 2 μmol/L. In contrast, mesangial proliferation induced by FGF-2 was unaffected by STI 571 (data not shown). Mesangial cells remained viable, as evidenced by trypan blue exclusion and the maintenance of normal mesangial cell appearance and, in particular, nuclear morphology.

Clinical characteristics

The administration of OX-7 IgG both with and without STI 571 was well tolerated by all experimental animals with no weight loss or abnormalities in hemoglobin, leukocyte, or platelet counts. Proteinuria was mildly increased in animals that received OX-7 IgG compared with control rats and was unaffected by STI 571 treatment (normal control, 2.1 ± 0.2 mg/24 hours; vehicle-treated Thy-1 nephritis, 13.4 ± 7.2 mg/24 hours; STI 571-treated Thy-1 nephritis, 16.4 ± 7.0 mg/24 hours). In PAS-stained sections, mesangial hypercellularity and increased mesangial matrix were noted in glomeruli of untreated rats. These pathological changes were not seen in rats receiving STI 571 (Fig. 2).

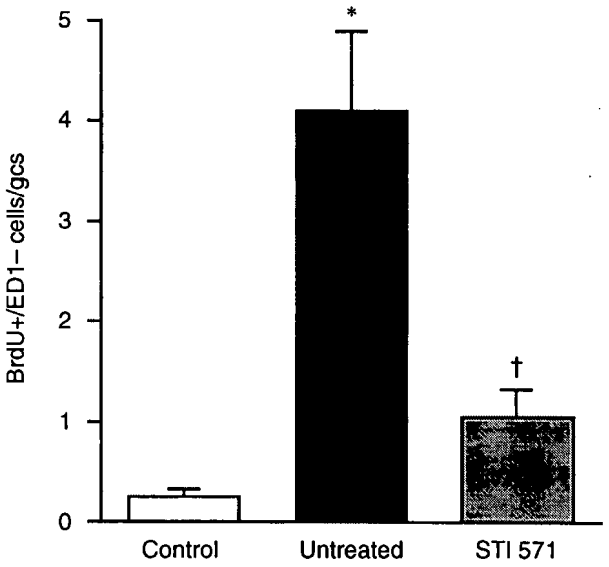

Fig. 5. Mesangial cell proliferation as assessed by the number of BrdU+/ED 1− cells (mean ± SEM) per glomerular cross-section (gcs) in 50 hilar glomeruli per animal. Mesangial cell proliferation was significantly attenuated by STI 571. *P < 0.001 vs. control; †P < 0.001 vs. untreated.

Glomerular cellularity and mesangial cell proliferation in rats with anti-Thy-1 nephritis

Animals with anti-Thy-1 nephritis displayed moderate glomerular hypercellularity when compared with control animals, as assessed by the nuclear counting; however, glomerular hypercellularity was significantly reduced in rats treated with STI 571 (Fig. 3). Mesangial cell (BrdU+ED 1−) proliferation was increased 16-fold compared with control rats and significantly reduced by treatment with STI 571 (Figs. 4 and 5). Similarly, the proportional area of glomeruli immunostained for α-smooth muscle actin indicating activated mesangial cells was also increased in untreated rats with anti-Thy-1 nephritis and significantly reduced by the administration of STI 571 (Figs. 6 and 7). In contrast, STI 571 had no effect on either glomerular macrophage numbers (Fig. 8A) or their proliferative activity (Fig. 8B).

Glomerular matrix accumulation

Marked accumulation of immunostainable type IV collagen was present in untreated rats with anti-Thy-1 nephritis and was significantly reduced by the administration of STI 571 (Figs. 6 and 7).

DISCUSSION

In the present study, blockade of PDGF receptor activity in experimental mesangial proliferative glomerulonephritis was associated with a reduction in the pathological hallmarks of this disease: hypercellularity and extracellu-

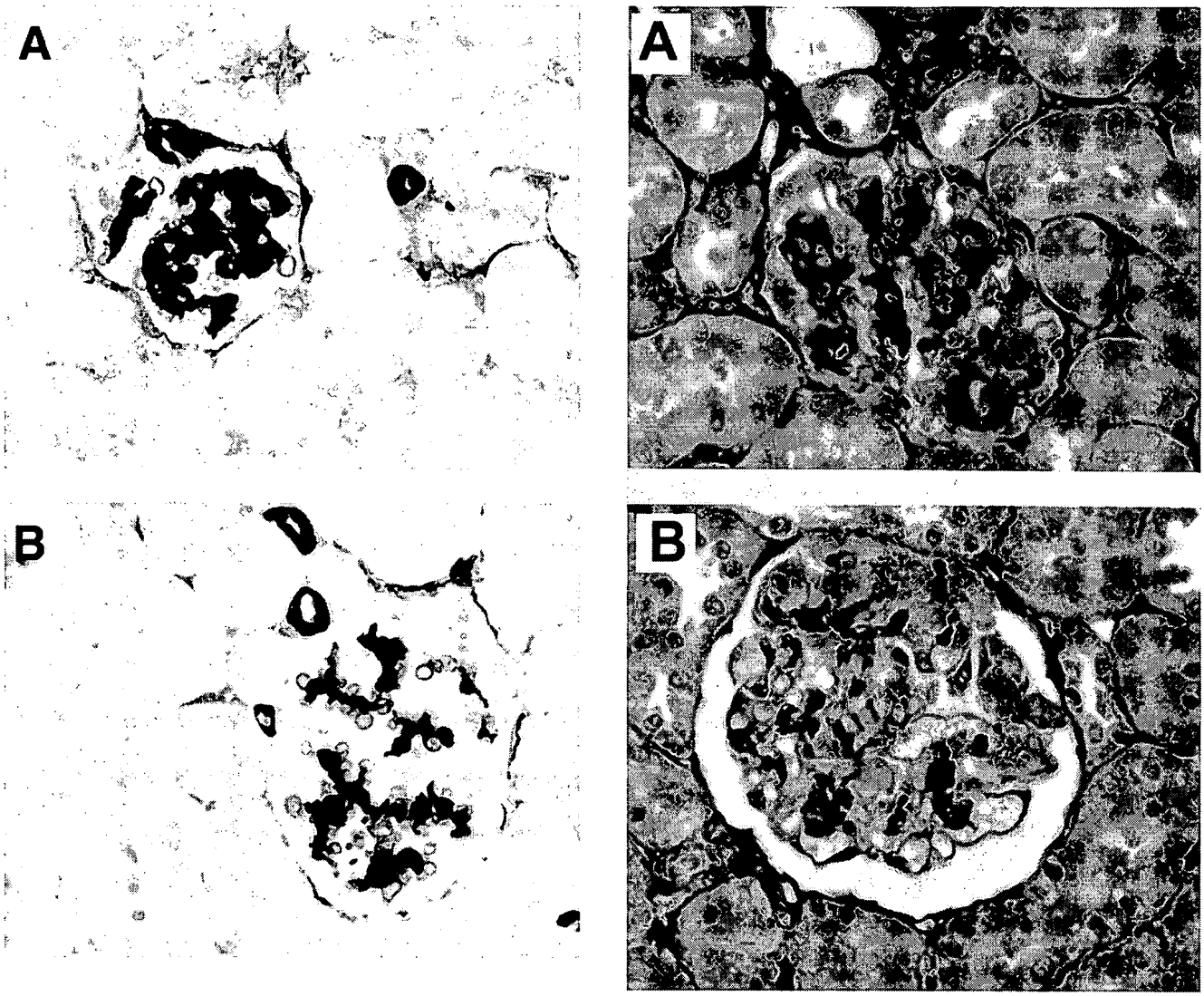

Fig. 6. Immunostaining for α-smooth muscle actin (left panel) and type IV collagen (right panel) in photomicrograph of kidney section from untreated (*A*), and STI 571-treated rats (*B*) with experimental mesangial proliferative glomerulonephritis. Magnification ×360.

lar matrix accumulation. Additionally, the antiproliferative effects of PDGF receptor blockade on mesangial cells were confirmed in vitro. These findings attest to the central role of PDGF in glomerular disease and provide a new therapeutic strategy for its treatment.

The major findings of the present study were the amelioration of mesangial hypercellularity and matrix accumulation by treatment with STI 571. Not only was PDGF-induced mesangial proliferation abrogated in vitro, but also in vivo, as shown by a reduction in cell nuclei and BrdU+ED 1− cells. In addition to these effects on cell proliferation, mesangial matrix expansion was also reduced by PDGF-RTK inhibition as evidenced in both PAS-stained sections and in tissues immunostained for the major glomerular extracellular matrix protein, type IV collagen. Such effects on extracellular matrix expansion may be particularly relevant to more chronic glomerular diseases in which mesangial matrix correlates closely with declining glomerular filtration rate [27–29]. In the present study, the reduction in extracellular matrix resulting from STI 571 administration may reflect changes in both direct and indirect PDGF related processes. For instance, while PDGF may directly stimulate extracellular matrix synthesis [30], it is also possible that the observed changes may have been a consequence of the fewer activated, synthetic-type, α-smooth muscle actin-positive mesangial cells [31]. In addition, the ability of PDGF to stimulate expression of the prosclerotic cytokine TGF-β in mesangial cells [12] suggests that such interactions may also contribute to the reduction in extracellular matrix observed in the present study.

The cellular actions of PDGF follow the binding of

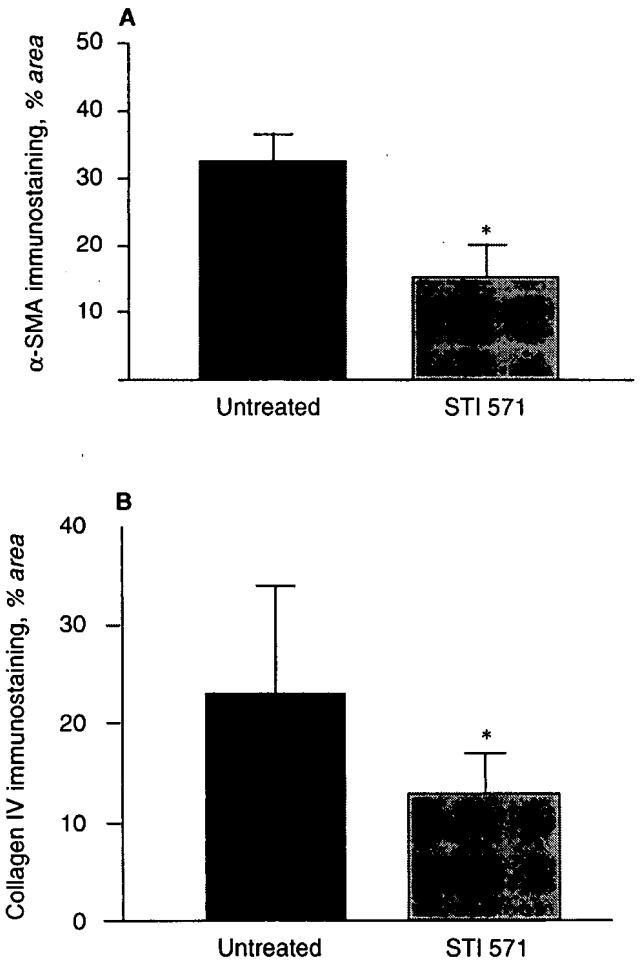

Fig. 7. Activated mesangial cells (*A*) and type IV collagen deposition (*B*) as assessed by the proportional area immunostained with α-smooth muscle actin and type IV collagen antibodies, respectively. Data are expressed as (mean ± SEM) per glomerular cross-section (gcs) in 50 hilar glomeruli per animal from untreated and STI 571–treated rats. $^*P < 0.05$.

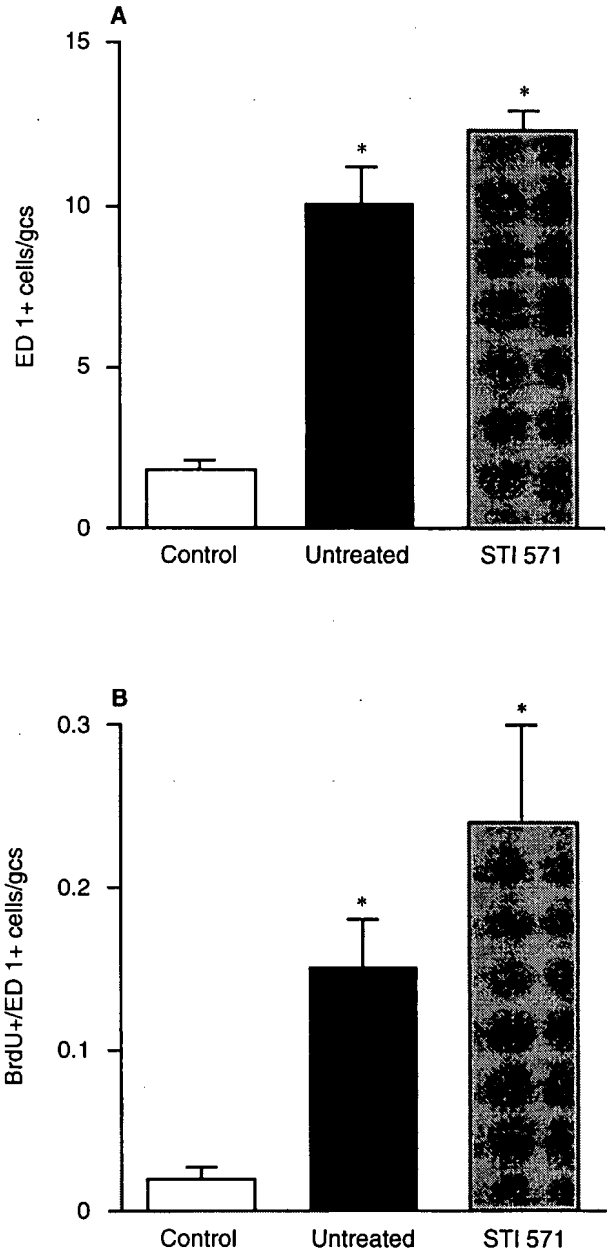

Fig. 8. Quantitation of macrophage numbers (ED 1+, *A*) and macrophage proliferation (*B*), as assessed by the number of ED 1+/BrdU+ cells (mean ± SEM) per glomerular cross-section (gcs) in 50 hilar glomeruli per animal. $^*P < 0.01$ vs. control.

ligand to the extracellular part of its receptor. This in turn leads to its dimerization and autophosphorylation ultimately leading to protein kinase C activation and consequent cell responses [6, 32]. STI 571 is a potent and selective inhibitor of the PDGF receptor (PDGF-R) and Abl protein tyrosine kinases [33]. While STI 571 is a potential treatment for Bcr-Abl–positive leukemias, its inhibitory effects on PDGFR activation suggests a potential role in nonmalignant diseases in which PDGF has been strongly implicated, such as glomerulonephritis. In contrast to its effects on the PDGF receptor, ligand-induced autophosphorylation of the receptors for epidermal growth factor, insulin-like growth factor I, and FGF are insensitive to STI 571, with IC_{50} >200-fold greater than for PDGF RTK [33]. Indeed, in the present study, STI 571 specifically inhibited PDGF-induced mesangial cell proliferation in a dose-dependent manner, but had no effect on FGF-2–induced mesangial ^{3}H-thymidine incorporation. By selectively targeting PDGF signaling, STI 571 administration in the in vivo setting resulted in cell-specific inhibition of mesangial cell, but not macrophage proliferation within the glomerulus.

Numerous studies have reported PDGF overexpression in a variety of human [34–38] and experimental glomerular diseases [39–41]. However, while there is substantial evidence suggesting that this overexpression of

PDGF is of major pathogenetic significance in glomerular disease [6], in order to establish a role for a specific cytokine in renal disease, it has been proposed that four criteria need to be satisfied [2]. These criteria, an adaptation of Koch's postulates, state the following: (*1*) the cytokine must have the relevant biological effect on target cells in vitro; (*2*) it should be expressed in disease states and correlate with the proposed biological effect; (*3*) its administration in vivo should reproduce the disease; and (*4*) blocking the cytokine should ameliorate the disease. While there is substantial experimental data to satisfy the first three criteria for PDGF's role in glomerular disease, only two previous studies have addressed the issue of PDGF blockade. In a study by Johnson et al, neutralizing anti–PDGF-B antibodies reduced glomerular cell proliferation and matrix accumulation in Thy 1.1 glomerulonephritis [42] attesting to the central role of PDGF in this disease model. More recently, Floege et al antagonized PDGF-B effects in rat anti–Thy-1.1 glomerulonephritis by reducing translation of PDGF-B mRNA with high-affinity nucleic acid aptamers [43]. The current findings further support a central role for PDGF in pathological mesangial proliferation. However, these previously identified means of targeting PDGF have limited applicability to the treatment of renal disease in humans. While neutralizing antibody administration is effective in rat models of glomerulonephritis [42], the use of large quantities of heterologous antibodies is not feasible in humans, and while the production of specific, high-affinity, monoclonal humanized antibodies is technically feasible, the paucity of such antibodies in clinical practice indicates the practical difficulties of this approach. Aptamer therapy is similarly effective in experimental disease but remains an uncertain possibility for human use. In contrast, STIs are already in clinical development [44, 45], with recent phase II studies of STI 571 in CML [46]. As in experimental mesangial proliferative glomerulonephritis, there is substantial evidence supporting a key pathogenetic role for PDGF in human glomerulonephritis characterized by mesangial proliferation such as IgA nephropathy [6], among the most common causes of end-stage renal failure in the world [47]. Thus, the ability to inhibit PDGF signal transduction may offer a new therapeutic approach to renal disease in humans.

In the present study, PDGF-RTK inhibition had no effect on glomerular macrophage infiltration and proliferation. These findings are consistent with studies of PDGF blockade with neutralizing anti-PDGF antibody [42] and aptamer-based PDGF antagonism [43], suggesting that monocyte/macrophage chemotaxis in this disease model is largely PDGF independent. Furthermore, the amelioration of mesangial cell proliferation and matrix accumulation without altering macrophage infiltration suggests that the macrophage may not have a direct role in the pathogenesis of this form of experimental glomerulopathy, although it may provide a source of excessive PDGF in this disease.

In summary, the strong association of both experimental and human mesangial proliferative glomerulonephritis with PDGF overexpression and the amelioration of the pathological findings in the present study by blockading PDGF receptor activity suggest the potential clinical utility of this approach as a therapeutic strategy in glomerular disease.

ACKNOWLEDGMENTS

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Reprint requests to Richard E. Gilbert, M.D., Ph.D., University of Melbourne, Department of Medicine, St. Vincent's Hospital, Victoria Parade, Fitzroy, Victoria, 3065, Australia.
E-mail: gilbert@medstv.unimelb.edu.au

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Brief Definitive Report

Inhibition of Mesangial Cell Proliferation and Matrix Expansion in Glomerulonephritis in the Rat by Antibody to Platelet-derived Growth Factor

By Richard J. Johnson,* Elaine W. Raines,‡ Jürgen Floege,* Ashio Yoshimura,* Pam Pritzl,* Charles Alpers,‡ and Russell Ross‡

*From the *Division of Nephrology, Departments of Medicine and ‡Pathology, University of Washington Medical Center, Seattle, Washington 98195*

Summary

Platelet-derived growth factor (PDGF), a potent mitogen for mesenchymal cells in culture, is expressed in vivo in a variety of inflammatory conditions associated with cell proliferation, including atherosclerosis, wound repair, pulmonary fibrosis, and glomerulonephritis. However, it is not known if PDGF mediates the fibroproliferative responses that characterize these inflammatory disorders. We administered neutralizing anti-PDGF IgG or control IgG to rats with mesangial proliferative nephritis. Inhibition of PDGF resulted in a significant reduction in mesangial cell proliferation, and largely prevented the increased deposition of extracellular matrix associated with the disease. This suggests that PDGF may have a central role in proliferative glomerular disease.

Mesangial cell proliferation and matrix expansion characterize many types of glomerulonephritis (GN). The observations that platelet-derived growth factor (PDGF) is a potent mitogen for mesangial cells in culture (1) and is expressed in both experimental and human GN in which mesangial cell proliferation occurs (2–5) suggest that this factor may have an important role in mediating these changes.

One model in which PDGF has been studied is the mesangial proliferative GN in rats induced by antibody to the Thy-1 antigen, which is expressed by mesangial cells (2). The model is characterized by an acute complement-dependent loss of mesangial cells with disruption of the mesangial matrix ("mesangiolysis") that is maximal at 24 h (2). The mesangial cell population, which is almost completely eliminated, undergoes a rebound proliferation that is accompanied by a marked upregulation of PDGF A and B chain mRNA in total glomerular RNA at 3 and 5 d after disease induction (2). When rats were depleted of either complement or platelets and were injected with anti-Thy-1 antibody, both the glomerular cell proliferation and increased glomerular PDGF expression were significantly inhibited (2, 3). However, despite demonstrating a strong association between glomerular PDGF expression and glomerular cell proliferation in mesangial proliferative GN, these studies do not determine whether PDGF plays a direct role in the pathogenesis of glomerular injury. We now report the effect of blocking PDGF in vivo in this model of nephritis utilizing a neutralizing polyclonal antibody to PDGF.

Materials and Methods

Experimental Protocol. Anti-Thy-1 GN was induced with goat anti-Thy-1 plasma in 150–200-g male Wistar rats (Simonsen, Gilroy, CA) as previously described (2). 8 h before the injection of anti-Thy-1 antibody, rats were injected with goat anti-PDGF IgG (60 mg/100 g body weight, i.p.) (n = 6) or equivalent quantities of nonimmune (control) goat IgG (n = 6) with repeated doses daily for 4 d. After disease induction, rats underwent renal biopsies at 2 and 4 d. Blood samples were collected for serum C3 levels (at 0, 2, and 4 d), leukocyte and platelet counts (day 4), and plasma anti-PDGF IgG levels (day 4, measured by ELISA [6]).

Anti-PDGF Antibody. The anti-PDGF IgG was raised in a goat immunized with PDGF purified from outdated human platelets and specifically neutralizes the mitogenic activity of rat PDGF and all dimeric forms of human PDGF (6).

Histology. The following antibodies were used for immunoperoxidase staining of methyl Carnoy's fixed, paraffin-embedded tissue: 19A2 (Coulter Immunology, Hialeah, FL), a mAb to the proliferating cell nuclear antigen (PCNA), which is a cell proliferation marker; ED-1 (Bioproducts for Science, Indianapolis, IN), a mAb to rat monocytes and macrophages; rabbit anti-rat collagen I and rabbit anti-rat laminin (Chemicon, Temecula, CA); rabbit anti-mouse collagen IV (Collaborative Research Inc., Bedford, MA); rabbit anti-mouse entactin (gift of A. Chung, Pittsburgh, PA); and rabbit anti-mouse heparan sulfate proteoglycan (gift of J. Couchman, Birmingham, AL) (2, 7). Immunofluorescence of snap-frozen tissue was also performed using FITC-conjugated rabbit anti-goat IgG and FITC-conjugated goat anti-rat C3 antibodies (Organon Teknika Corp., West Chester, PA) (8). Histological changes were quantitated as previously described (2, 7). PDGF B

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chain mRNA was detected in formalin-fixed tissue using a digoxigenin-labeled antisense cRNA probe and quantitated as previously described (3).

Statistics. Values are expressed as mean ± SD. The one-tailed student's *t* test was used to test the hypothesis that anti-PDGF treatment would reduce cell proliferation, total glomerular cellularity, or matrix expansion relative to controls.

Results and Discussion

In this study, neutralizing anti-PDGF IgG or control IgG was administered to rats with mesangial proliferative GN. Two time points were selected for study, representing the day of initial proliferation (day 2) and the day of peak proliferation (day 4). Later time points were not studied due to the concern that the rats would develop autoantibodies to the administered goat IgG.

Anti-PDGF IgG treatment was well tolerated, and serum C3 levels (measured at 0, 2, and 4 d) and platelet and leukocyte counts (at 4 d) were normal and not different from controls. Whereas anti-PDGF IgG levels were undetectable in control rats, plasma anti-PDGF IgG levels in treated rats were 4.6 ± 0.7 mg/ml at day 4, concentrations that are 20–30 times that required to inhibit the mitogenic activity of PDGF on rat smooth muscle cells in vitro (6).

Previous studies have demonstrated that the initial injury (i.e., mesangiolysis) in this model is dependent on delivery and binding of anti-Thy-1 antibody to the mesangial cell followed by complement activation (9). Anti-PDGF IgG treatment did not prevent this initial injury, as both control and anti-PDGF IgG–treated rats had equivalent mesangiolysis (2.6 ± 0.5 vs. 2.9 ± 0.2, respectively, scale of 0–4+; p = NS) with an equal reduction in total glomerular cellularity at day 2 (Table 1). Anti-PDGF IgG treatment also did not inhibit the glomerular macrophage infiltration at either day 2 (9.1 ± 2 vs. 9.2 ± 1) or day 4 (8.0 ± 2 vs. 8.8 ± 1 ED-1$^+$ cells/glomerular cross-section in control vs. anti-PDGF IgG–treated rats, respectively).

Table 1. *Effect of Anti-PDGF IgG Treatment on Total Glomerular Cellularity and Proliferating (PCNA$^+$) Cells in Mesangial Proliferative GN.*

	Total cells	Proliferating (PCNA$^+$) cells
Normal	77 ± 1.8	0.9 ± 0.2
Mesangial proliferative GN, day 2		
Control	53 ± 5	10.4 ± 1
Anti-PDGF	53 ± 6	10.0 ± 2
Mesangial proliferative GN, day 4		
Control	89 ± 13	13.4 ± 4
Anti-PDGF	77 ± 10*	5.7 ± 1‡

Values are expressed as the mean number ± SD of cells per glomerular cross-section. For comparison, values for normal Wistar rats (n = 6) are shown (2).
* p <0.05 relative to control rats.
‡ p <0.005 relative to control rats.

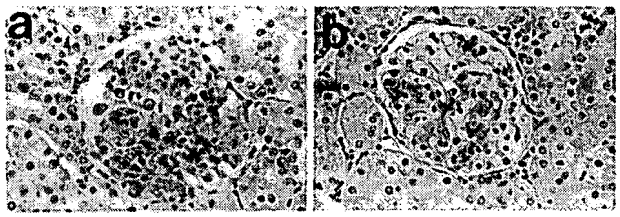

Figure 1. Compared with control rats with mesangial proliferative GN (*a*), anti-PDGF IgG–treated rats with GN (*b*) had significantly less glomerular cellularity at day 4 (periodic acid/Schiff reagent with hematoxylin counterstain, ×240).

Effect on Glomerular Cell Proliferation. The initial glomerular cell proliferation (i.e., PCNA$^+$ cells) was not affected by anti-PDGF IgG treatment (Table 1). In contrast, at day 4, during the peak phase of cell proliferation, a 57% reduction in cell proliferation was observed ($p < 0.005$), and was associated with a significant reduction in total glomerular cellularity as compared with controls (Table 1 and Fig. 1). Most of the proliferating cells in this model have been shown to be mesangial cells (2, 8), and this was supported in the current study in which >85% of the PCNA$^+$ cells in both groups excluded the monocyte-macrophage marker, ED-1, by double immunolabeling.

One concern was that anti-PDGF IgG treatment might result in significant PDGF/anti-PDGF immune complexes in glomeruli that would prolong the mesangiolysis and delay the proliferative response. However, both control and anti-PDGF IgG–treated rats had equivalent mesangial staining of IgG at 2 and 4 d (2+, scale of 0–4+) with negative staining of C3 by immunofluorescence. Similarly, incubation of both resting and proliferating mesangial cells in culture with anti-PDGF IgG (5 mg/ml) resulted in no significant ^{51}Cr release compared with control IgG in the presence of complement (data not shown). Finally, injection of equivalent doses of anti-PDGF IgG into normal rats (n = 2) as used in the experimental study resulted in no detectable mesangial injury in biopsies obtained at days 2 and 4.

The observation that mesangial cell proliferation was reduced by anti-PDGF IgG treatment at day 4 but not at day 2 has several potential interpretations, including: (*a*) the possibility that numerous growth factors, including PDGF, are involved in the initial cell proliferation such that mesangial cells are maximally stimulated even when PDGF is inhibited; (*b*) the possibility that inadequate anti-PDGF IgG was present locally to block PDGF before its binding to the mesangial cell at day 2; and (*c*) the possibility that responsiveness to PDGF requires upregulation of the PDGF receptor. PDGF receptor β subunit expression is known to be upregulated in this disease (2), and in this study, PDGF receptor β subunit immunostaining was markedly increased in glomeruli at day 4 relative to day 2 (data not shown).

Other possible growth regulatory molecules that could mediate the initial mesangial cell proliferation include factors

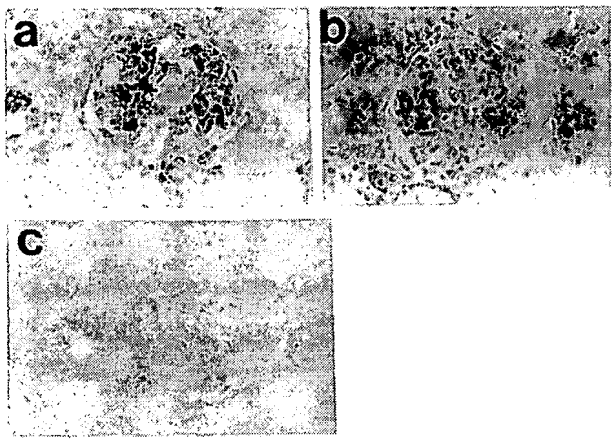

Figure 2. Whereas the expression of PDGF B chain mRNA was often diffuse in glomeruli of control rats with mesangial proliferative GN (*a*), in anti-PDGF IgG–treated rats with GN, expression was often segmental (*b*). Hybridization with a sense probe for PDGF B chain mRNA was always negative (*c*) (×240).

released by platelets, since thrombocytopenic animals show a decrease in mesangial cell proliferation in this model at day 3 (8) and basic fibroblast growth factor (bFGF), which is released by a variety of damaged cells, and is a mitogen for mesangial cells in culture (10). Recently, we have shown that mesangial cells produce bFGF, and release bFGF during acute mesangiolysis (i.e., the first 24 h) in this model (J. Floege et al., manuscript submitted for publication). This may also be relevant to the PDGF-mediated response, given that bFGF will induce both PDGF expression by mesangial cells in culture (10) and an increase in PDGF receptors on smooth muscle cells (our unpublished observations).

Glomerular Source of PDGF. Although the source of the glomerular PDGF may partially originate from platelets, activated macrophages, or endothelial cells, our previous studies (2, 3) suggest that most of the PDGF is expressed by mesangial cells, where it may function as an autocrine growth factor.

Table 2. *Effect of Anti-PDGF IgG Treatment on Extracellular Matrix Accumulation in Mesangial Proliferative GN*

	Normal	Control	Anti-PDGF
Type IV collagen	0.9 ± 0.8	3.1 ± 0.7	2.1 ± 0.4*
Type I collagen	0.03 ± 0.02	2.5 ± 0.6	1.6 ± 0.4*
Laminin	0.8 ± 0.4	2.6 ± 0.6	1.8 ± 0.3*
Entactin/nidogen	0.2 ± 0.1	2.5 ± 0.7	1.6 ± 0.4*
Heparan sulfate proteoglycan	1.8 ± 0.5	3.1 ± 0.6	2.6 ± 0.6

Semiquantitative immunohistochemical scores for various ECM components (scale of 0–4+ [7]) in the mesangium of rats with mesangial proliferative GN that had been treated with nonimmune IgG (control, $n = 6$) or with anti-PDGF IgG ($n = 6$). For comparison, the values in normal Wistar rats ($n = 6$) are shown.
* $p < 0.01$ vs. control rats.

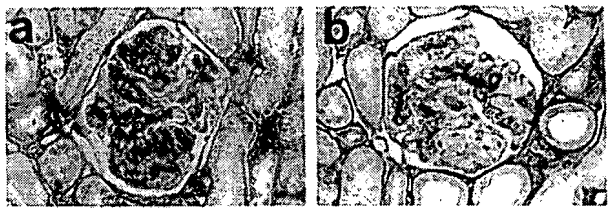

Figure 3. A diffuse increase in laminin was present in the mesangium of control rats with GN (*a*), and was significantly reduced in rats with GN that had received anti-PDGF IgG (*b*) (×240).

We therefore performed in situ hybridization to determine the effect of anti-PDGF IgG treatment on PDGF B chain mRNA expression at day 4. An increase in PDGF B chain mRNA could be detected in mesangial regions in both control and anti-PDGF IgG–treated rats (Fig. 2). However, the amount of PDGF B chain mRNA in the glomeruli was lower in anti-PDGF IgG–treated animals (1.68 ± 0.4 vs. 1.24 ± 0.2 in control vs. anti-PDGF-treated rats, scale of 0–3+, one-tailed Student's *t* test; $p < 0.05$ [3]). The reduction in glomerular expression of PDGF B chain mRNA in the anti-PDGF IgG–treated rats is consistent with an inhibition of mesangial cell proliferation and an interruption of PDGF-mediated induction of its own expression in mesangial cells (10).

Effect of Anti-PDGF IgG Treatment on Extracellular Matrix (ECM) Accumulation. Previous studies have demonstrated that in this model mesangial cell proliferation is accompanied by an expansion of several ECM proteins in the mesangium (7, 11). In this study, control rats with anti-Thy-1 GN also had a diffuse increase in glomerular staining at day 4 for various ECM proteins, including types I and IV collagen, laminin, and entactin (Table 2). In contrast, glomeruli from anti-PDGF IgG–treated rats with GN showed significantly less, and often only segmental, increases in staining (Table 2 and Fig. 3).

The reduction in immunostaining for the various ECM components in anti-PDGF IgG–treated rats may reflect, in part, the reduction in mesangial cell number. Alternatively, anti-PDGF treatment may be affecting TGF-β production within the glomeruli. PDGF induces mesangial cells to express TGF-β (12). TGF-β, in turn, induces mesangial cells to produce a variety of ECM components (13, 14). TGF-β is also increased in glomeruli of rats with anti-Thy-1 GN, and treatment of rats with anti-Thy-1 GN with anti-TGF-β antibody inhibits expansion of the mesangial matrix (11). Thus, it is possible that the beneficial effects of anti-PDGF treatment on ECM expansion in anti-Thy-1 GN may reflect inhibition of PDGF-mediated stimulation of mesangial cell production of TGF-β.

In conclusion, the current study provides the first direct in vivo evidence for a role for PDGF as a growth stimulatory molecule in GN. Treatment of rats with mesangial proliferative GN with a neutralizing anti-PDGF antibody significantly reduced mesangial cell proliferation and matrix expansion at 4 d. The observation that the inhibition was only partial (i.e., 60%) suggests that other growth factors may be involved in this proliferative response, or that there was insufficient antibody available at the cellular level to effect a total response.

The fact that this degree of proliferation and matrix expansion could be reduced by inhibiting a single growth factor suggests that PDGF may play a crucial role in progressive glomerular injury.

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Address correspondence to Richard J. Johnson, Division of Nephrology, RM-11, Department of Medicine, School of Medicine, University of Washington, Seattle, WA 98195.

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